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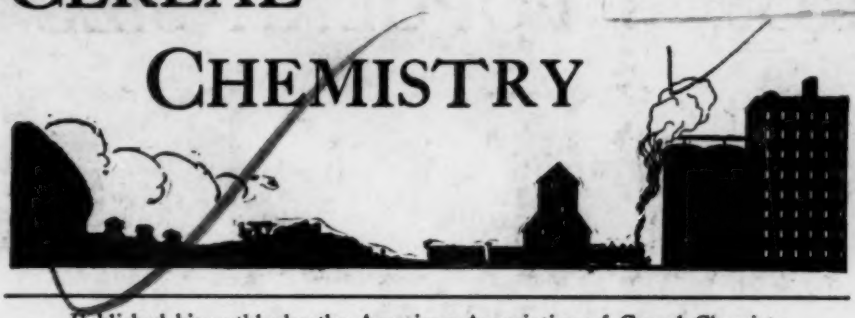

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EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

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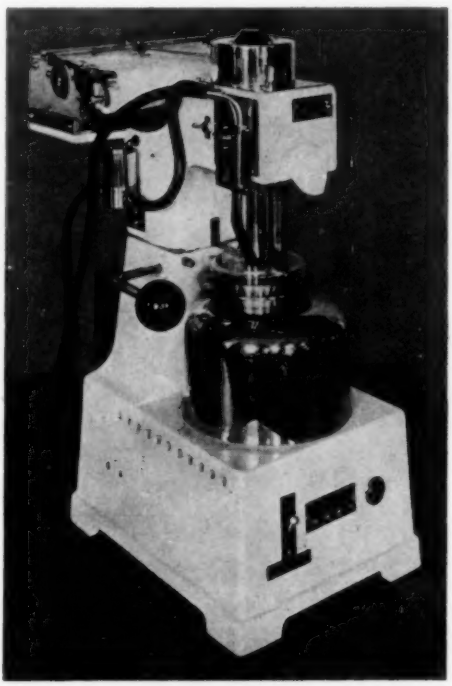

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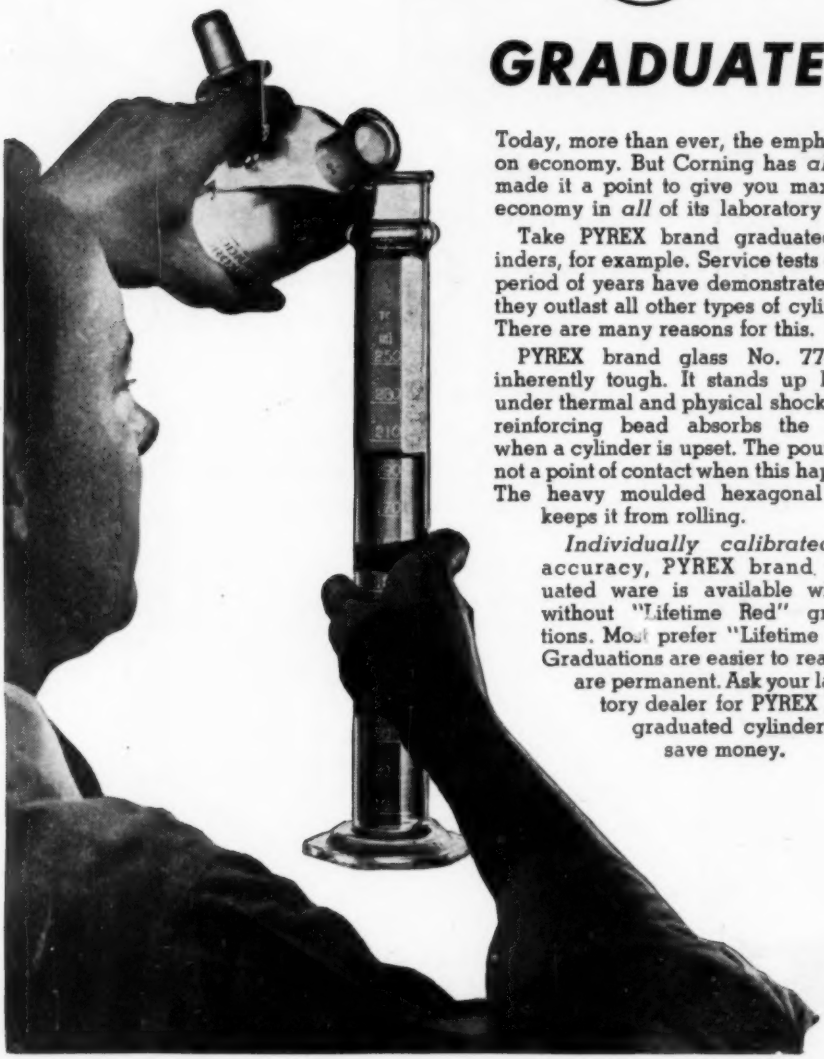

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CEREAL CHEMISTRY

VOL. XXVI SEPTEMBER, 1949 NO. 5

EFFECT OF SURFACE-ACTIVE AGENTS ON THE SOFTNESS AND RATE OF STALING OF BREAD [1]

E. C. EDELMANN and W. H. CATHCART [2]

ABSTRACT

The relative effectiveness in bread of 24 different surface-active agents as emulsifiers or "softeners" was determined. The results of compressibility tests and organoleptic examinations made on bread showed that five of the surface-active agents were more effective than the others. They were glyceryl monostearate, glyceryl oleostearate, polyoxyethylene stearate, sorbitan monostearate, and polyoxyethylene sorbitan monostearate.

Additional compressibility and organoleptic data obtained from commercial-scale baking tests of the five more effective emulsifiers showed that polyoxyethylene stearate was the most effective bread softener of the emulsifiers investigated. It also brightened the crumb color and improved the grain and texture of bread. The four other selected agents had similar bread-improving properties, but it was necessary to use larger quantities. Bread containing polyoxyethylene stearate had a lower rate of increase in crumb firmness upon storage than the control (no emulsifier added) bread.

Bread containing polyoxyethylene stearate or glyceryl monostearate when stored at 10°C. was not as soft from 12 to 96 hours after baking as control bread stored at approximately 28°C. However, the difference in degree of softness between control bread and those containing an emulsifier was greater at 10°C. than at 28°C.

Control bread 48 hours old was refreshened by heating to 60°C. in a radio-frequency field. It was not as soft at any time from 12 to 72 hours after refreshening as unheated bread of the same age containing polyoxyethylene stearate, glyceryl monostearate, or glyceryl oleostearate. Refreshened loaves containing emulsifiers had a greater rate of increase in crumb firmness upon storage than the respective unheated loaves. The rate was the same for refreshened and unheated control bread.

The addition of emulsifiers did not affect the rate of moisture loss in bread during a 96-hour staling period.

The homogenization at 1,000 and 3,000 p.s.i. of the sugar, nonfat dry milk solids, yeast food, and part of the water was ineffective as a method of making soft bread having improved crumb color, texture, and grain. Homogenizing glyceryl monostearate with these ingredients did not improve its effect.

[1] Manuscript received October 25, 1948.

[2] The Great Atlantic and Pacific Tea Company, National Bakery Division, New York 17, N. Y.

During the past several years the baking industry has become increasingly conscious of surface-active agents. Claims have been made that various compounds having surface-active properties are effective adjuncts in producing soft bread and in delaying the staling of baked products. Several workers have evaluated certain of these compounds. Carlin, Hopper, and Thomas (2) reported that a relationship exists between increasing firmness of the crumb and staling phenomena; that increasing firmness in bread crumb is a linear function of storage time; and that modified fats affect the slope of the staling curve. Favor and Johnston (5) reported that polyoxyethylene stearate is a crumb-softener with the ability to decrease the rate at which bread crumb becomes hard with age. Freilich (6) found that polyoxyethylene stearate decreased the staling rate of bread crumb, as measured by a compressibility method (but not as measured by the farinograph). This paper reports an investigation of the effect on bread of 24 different surface-active agents in general and five in detail.

As reviewed by Cathcart (3) and Geddes and Bice (7) bread stales rapidly below 60°C. and above 0°C. Work was done to learn whether the addition of emulsifiers would nullify the firming effect a lower temperature (10°C.) has on bread crumb. Such information would have practical application in areas where bread is transported at low temperatures.

Bread that has staled may be refreshened by heating to 60°C. or above (3, 7). It was of interest to study the effect emulsifiers have on bread refreshened by heat in a radio-frequency field. Moisture loss rates of refreshened and unheated bread were determined.

It has been stated that high-pressure homogenization of certain of the ingredients will keep bread soft and delay staling. Because like claims have been made for emulsifiers and the process of homogenization, their effect on bread has been compared.

Materials and Methods

Emulsifiers Tested. Table I lists the 24 different surface-active compounds investigated with a rating of their relative effectiveness in bread. This paper refers to the surface-active agents reported as bread emulsifiers or "softeners," although many of these compounds had been previously listed as suitable for all surface-active needs (8). During the preliminary survey of these surface-active agents the possible toxicity of many of them was ignored. After the initial testing all but five of the compounds were eliminated because of their ineffectiveness or because they imparted a foreign flavor to bread. The five selected compounds, glyceryl monostearate, glyceryl oleostearate, polyoxyethylene stearate, sorbitan monostearate, and polyoxyethylene

sorbitan monostearate, were studied further. Although polyethylene glycol (mono) laurate was rated excellent in effectiveness it was eliminated because it is closely related chemically to polyoxyethylene stearate and is no more effective. Glyceryl monostearates containing different percentages of mono-compound, manufactured with and without the addition of soap, dry and emulsified with water, were tested. Polyoxyethylene stearate manufactured by two different

TABLE I

SURFACE-ACTIVE AGENTS TESTED IN BREAD

Surface-active agent	Effectiveness in bread	Comments
Polyoxyethylene stearate	Excellent	—
Polyethylene glycol (mono) laurate	Excellent	—
Glyceryl (mono) oleate	Good	—
Glyceryl monostearate	Very good	—
Lecithin	Good	—
2-amino-1-butanol	Good	—
2-amino-2-methyl-1-propanol	Good	Imparts foreign odor to bread
Tris (hydroxymethyl) aminomethane	Poor	Ineffective in bread
2-aminoethyl ethanolamine	Fair	Alkaline reaction in bread
Diethylethanolamine	Good	Imparts foreign odor to bread
Monoethanolamine	Poor	Ineffective in bread
Morpholine	Fair	—
Sodium salt of higher secondary alkyl sulfate	Fair	—
Triethylene tetramine	Poor	Ineffective in bread
Diethylenetriamine	Fair	Imparts foreign odor to bread
Diethanolamine	Fair	Imparts foreign odor to bread
Triethanolamine	Fair	—
Polyoxyethylene ether of partial lauric acid ester	Good	—
Polyoxyethylene ether of partial palmitic acid ester	Good	—
Polyoxyethylene ether of partial stearic acid ester	Good	Used in mixture X
Polyoxyethylene ether of partial oleic acid ester	Good	—
Sorbitan monostearate	Good	Used in mixture X
Propylene glycol monostearate	Good	—
Glyceryl oleo stearate	Very good	—
Mixture X (88% sorbitan monostearate, 12% polyoxyethylene sorbitan monostearate)	Very good	—

processes and several brands of glyceryl oleostearate were tested. A mixture "X," composed of 88% sorbitan monostearate and 12% polyoxyethylene sorbitan monostearate, was made from two of the compounds selected for study.

Polyoxyethylene stearate and glyceryl oleostearate are plastic at 28°C., resemble firm lard, and incorporate readily with the other bread ingredients. Polyoxyethylene stearate was used alone, in a flour dispersion (75% flour plus 25% active ingredients), and emulsified

with 33.3%, 50%, and 75% of water. Neither polyoxyethylene stearate nor glyceryl oleostearate increased the water-absorbing properties of the dough. Glyceryl monostearate in flake and bead form was tested; it was employed dry and in water emulsions containing 25% and 33.3% emulsifier. Glyceryl monostearate increased water absorption when it was added to a dough. Mixture "X" was dispersed in flour (75% flour plus 25% active ingredients). The active ingredients of mixture "X" did not change dough absorption.

Baking Procedures. In both laboratory and commercial baking tests the sponge and dough process was used. The flour used throughout was hard winter wheat patent. The emulsifiers, the lard, and 60% of the total flour were included in the sponge. In all series a control made without an emulsifier was included. The laboratory baking test loaves weighed 16 oz. and yielded 18 slices approximately 0.4 inch thick. Commercial test loaves weighed 24 oz. and gave 25 slices approximately 0.5 inch thick. All loaves were sliced in a commercial type machine, wrapped, and weighed 90 minutes after baking. Laboratory-baked bread was wrapped in 300 MSAT [3] cellophane and commercial bread in opaque waxed paper. Except for the preliminary screening of the emulsifiers and the homogenization study, tests were made on bread produced from commercial size (1,000 lb.) doughs.

The selected emulsifiers were added to dough in quantities of 0.25%, 0.5%, 0.75%, 1.0%, 1.5%, and 2.0% (based on weight of flour as 100%). This was approximately equivalent to 0.156%, 0.312%, 0.468%, 0.624%, 0.936% and 1.25% of emulsifier in the baked bread. All were tested to find whether their effectiveness differed when added to the sponge or to the dough stage.

Organoleptic Tests. During the earliest part of the work the softness of bread was determined by squeezing the loaf by hand, feeling the texture of the crumb, eating the bread, and mashing the cut surface of the loaf with the fist and noting its resiliency. The organoleptic examinations were made by a panel usually consisting of five laboratory workers, all of whom were familiar with bread-quality factors. Test bread was compared with a control of the same age. Preferences were not scored numerically, but relative ratings were noted.

Compressibility Procedure. In the later studies the Bloom Gelometer, having a modified plunger of 1 inch diameter, was used, in addition to the organoleptic tests, to determine the compressibility of bread crumb (2). The numerical values obtained with this instrument made possible the plotting of graphs illustrating the degree and rate of change in crumb firmness. The Bloom Gelometer measures

[3] M—Moistureproof; S—Heat Sealing; A—Anchored (Water Resistant); T—Transparent (Uncolored).

the weight in grams required to cause a circular plunger, 1 inch in diameter, to depress the surface of a 1 inch (25.4 mm.) thickness (i.e., two slices 0.5 inch thick) of bread by 4 mm. The firmer the crumb the greater the weight required to depress the surface 4 mm. Compressibility tests were also made with the A.S.T.M. Penetrometer, but it was found that the Bloom Gelometer has less shearing effect on the crumb than the penetrometer.

When making compressibility tests the end slices of the loaf were not used. The crumb surface of every second slice was tested and two slices were placed together for testing on the instrument. The plunger was in contact with the crumb at a distance approximately 1 inch from the bottom and side crust of the slice. The compressibility value obtained was the average of eight different compressibility tests for the 16-oz. and 10 for the 24-oz. loaf. In most series compressibility tests were made 12, 24, 48, 72, and 96 hours after baking. In many the first test was made 90 minutes after baking, then at 12 hours, and every 12-hour period thereafter until 96 hours had elapsed. In some series the time was extended to 120 hours.

Storage at Different Temperatures. The breads containing polyoxyethylene stearate and glyceryl monostearate and a control were stored, 90 minutes after baking, at room temperature (approximately 28°C.) and at 10°C. Compressimetric and organoleptic tests were made during a 96-hour period. The bread stored at 10°C. was tested immediately upon removal from storage and also after being warmed to room temperature. (Six hours were required for the temperature to rise from 10°C. to 28°C.)

Refreshening of Bread. The breads containing polyoxyethylene stearate, glyceryl monostearate, and glyceryl oleostearate and a control were stored at room temperature (approximately 28°C.) for 48 hours and then refreshened by heating in a radio-frequency field. The interior of the bread was heated to 60°C. in a 25 megacycle field. This method was used so that the bread could be kept wrapped during heating, thereby preventing undue moisture loss. The same general method as was used by Cathcart, Parker, and Beattie (4) was employed. Compressibility and organoleptic tests were made after the bread had cooled to 30°C. (6 hours after heating); and at 72, 96, and 120 hours after baking.

Moisture Retention. The moisture content of bread was determined to learn the effect of the emulsifiers on the moisture retention of bread. Ninety minutes after baking and immediately after slicing and wrapping, the bread was weighed to 0.1 g. The loaves tagged for the determination of moisture were reweighed before each compressibility test period. After the final test period the moisture content of the bread

was determined (1). The moisture content of the bread at the time of each compressibility test was determined by using as an initial weight that obtained at the time of the compressibility test.

Homogenization. The sugar, nonfat dry milk solids, yeast food, and part of the water were homogenized at 1,000 and 3,000 p.s.i. in a Manton-Gaulin two-stage homogenizer. Other emulsions were made containing glyceryl monostearate in addition to these ingredients.

Results and Discussion

Effectiveness of Emulsifiers. The average results of organoleptic tests and the compressibility values obtained on more than 100 different laboratory and commercial baking tests showed that polyoxyethylene stearate kept bread softer for a longer period of time than bread made without it. It also brightened the crumb color slightly and improved the grain and texture of bread. With the bread formula used, 0.5% polyoxyethylene stearate was sufficient to give the maximum softening and improving effect. The same quantities of glyceryl monostearate and glyceryl oleostearate were not as effective as polyoxyethylene stearate. However, both were effective in keeping bread softer than

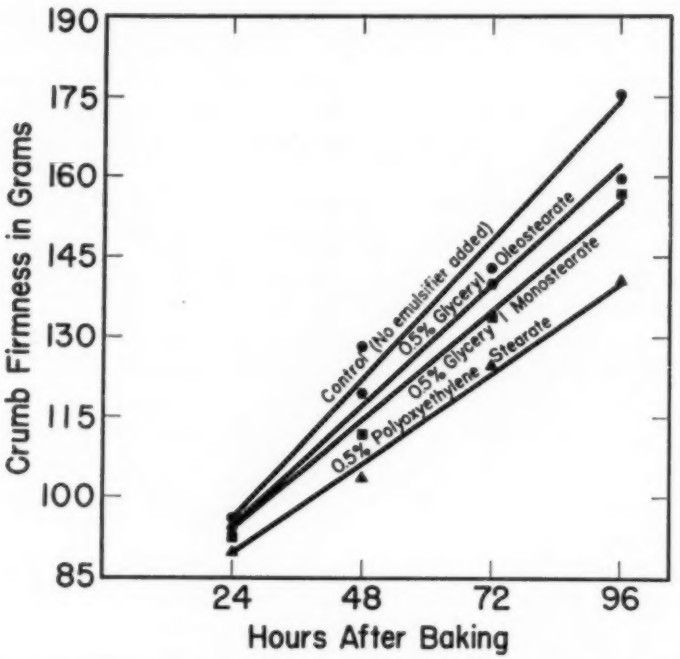

Fig. 1. Effect of 0.5% (flour basis) of various emulsifiers on the degree and rate of increase in crumb firmness using the Bloom Gelometer. Glyceryl oleostearate and polyoxyethylene stearate, both in plastic form (100% active), and 1.5% of a water emulsion of glyceryl monostearate (33.3% active) were used.

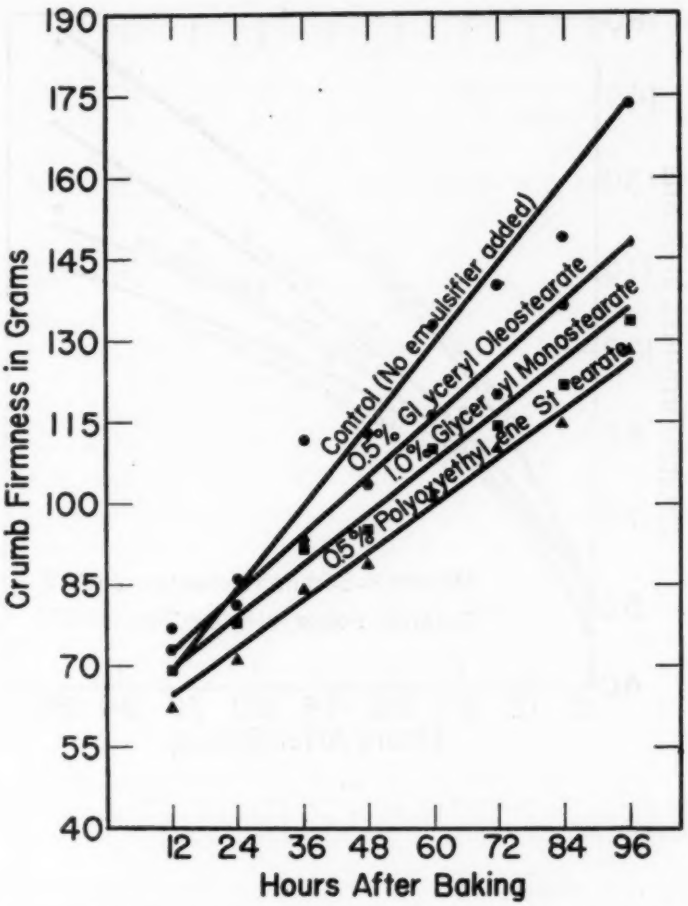

Fig. 2. Effect of emulsifiers on the degree and rate of increase in crumb firmness using the Bloom Gelometer. Glyceryl oleostearate and polyoxyethylene stearate, both in plastic form (100% active), and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

the control. Bread containing 1.0% glyceryl monostearate approached but did not equal the softness of crumb of bread containing 0.5% polyoxyethylene stearate (Figs. 2 and 3). Mixture "X" was not as effective as the same quantities of polyoxyethylene stearate, glyceryl monostearate, or glyceryl oleostearate but made bread softer than the control. Polyoxyethylene stearate was equally effective whether used in a plastic condition or dispersed in flour or water. Glyceryl monostearate showed almost the same bread softening properties when added dry to the dough that it did when premixed (emulsified) with water. Glyceryl monostearate was most effective when twice its weight in extra water was added to the dough. The same relative rating as emulsifiers was obtained for polyoxyethylene stea-

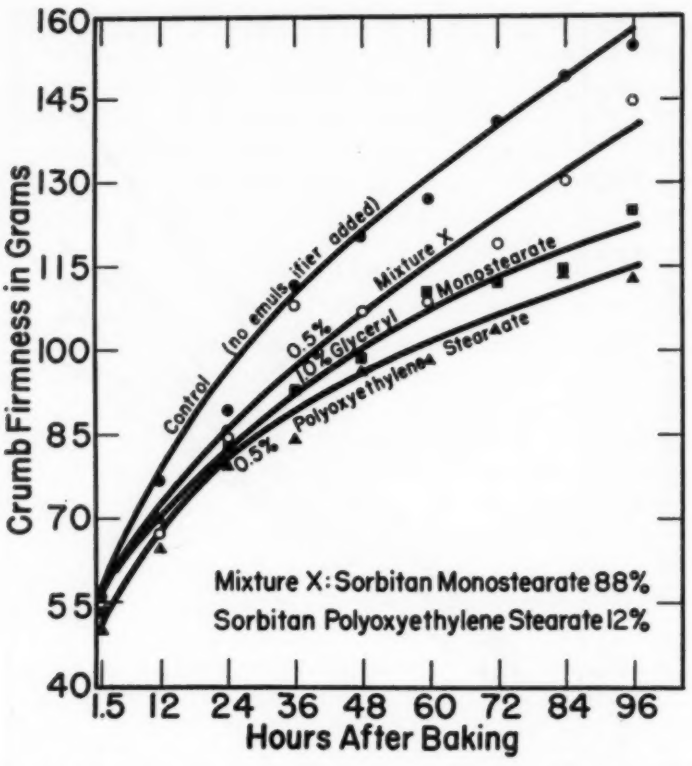

Fig. 3. Effect of emulsifiers on the degree and rate of increase in crumb firmness using the Bloom Gelometer. Polyoxyethylene stearate in plastic form (100% active), 3.0% of a water emulsion of glyceryl monostearate (33.3% active), and 2.0% of a flour dispersion of mixture X (25% active) were used.

rate, glyceryl monostearate, glyceryl oleostearate, and mixture "X" whether they were added to the sponge or to the dough stage. Polyoxyethylene stearates manufactured by two different processes were equally effective as bread softeners and improvers.

Favor and Johnston (5) reported that polyoxyethylene stearate, in quantities up to 2.0% of the flour weight, had no significant effect on bread doughs during processing or on the physical character of the bread other than its softness and tenderness. It was noted during the work reported here that the addition of 0.5% of this material to dough improved its machining properties. As mentioned previously, it also brightened the crumb color and improved the grain and texture of bread. When processed and baked under comparable conditions, bread containing polyoxyethylene stearate had a slightly darker crust color than the control. The emulsifiers had little effect on bread volume, and in any particular series, the loaf volumes were the same, within experimental error.

Figs. 1, 2, and 3 illustrate the effect of polyoxyethylene stearate, glyceryl monostearate, glyceryl oleostearate, and mixture "X" on bread crumb firmness (compressibility) during 96-hour staling periods. These are typical curves obtained on particular days and are representative of the results obtained in many series. Fig. 3 shows that 90 minutes after baking, differences in compressibility value are slight; and at the beginning the curves are not straight lines. The difference in the firmness (compressibility) between bread containing an emulsifier and the control is very small within a few hours after baking but becomes significant by 48 hours. Differences in the degree of softness were first observable, by organoleptic methods, 12 hours after baking.

Some workers have placed emphasis on the slope of the curves obtained from compressibility data, believing that the slope differentiates between the effect of various emulsifiers on the rate at which bread crumb becomes firm. Only after repeated tests may such conclusions be drawn, because the results of baking tests are affected by many complex factors. Differences in fermentation time, temperature, and humidity, in ingredients, in scaling weight, and in baking and cooling conditions will affect compressibility data. As a result of the many tests conducted, typical examples of which are reported here, it was found that bread containing 0.5% polyoxyethylene stearate had a lower rate of increase in crumb firmness upon storage than control bread. The slope of the curves obtained from bread containing 0.5% of glyceryl monostearate, glyceryl oleostearate, and mixture "X" indicated a rate of increase in crumb firmness which was intermediate between the control bread and that containing polyoxyethylene stearate. The differences in rate for equal quantities of glyceryl monostearate, glyceryl oleostearate, and mixture "X" are not believed significant; i.e., the differences were not easily observed when checked by organoleptic tests. However, all the emulsifiers selected for study kept bread softer than the control.

Effect of Different Storage Temperatures. Fig. 4 illustrates the effect of storage at 10°C. and at room temperature (approximately 28°C.) on the degree and rate of increase in crumb firmness during a 96-hour staling period. From 12 hours to 96 hours after baking all bread stored at 10°C. and warmed to room temperature before compressibility tests were made had a firmer crumb than the respective bread stored continuously at room temperature. In all cases the firmness of crumb was exaggerated when bread stored at 10°C. was tested immediately upon removal from storage. From these results it is concluded that the increased firmness effect of low temperatures (10°C.) on bread crumb cannot be compensated for by the addition of

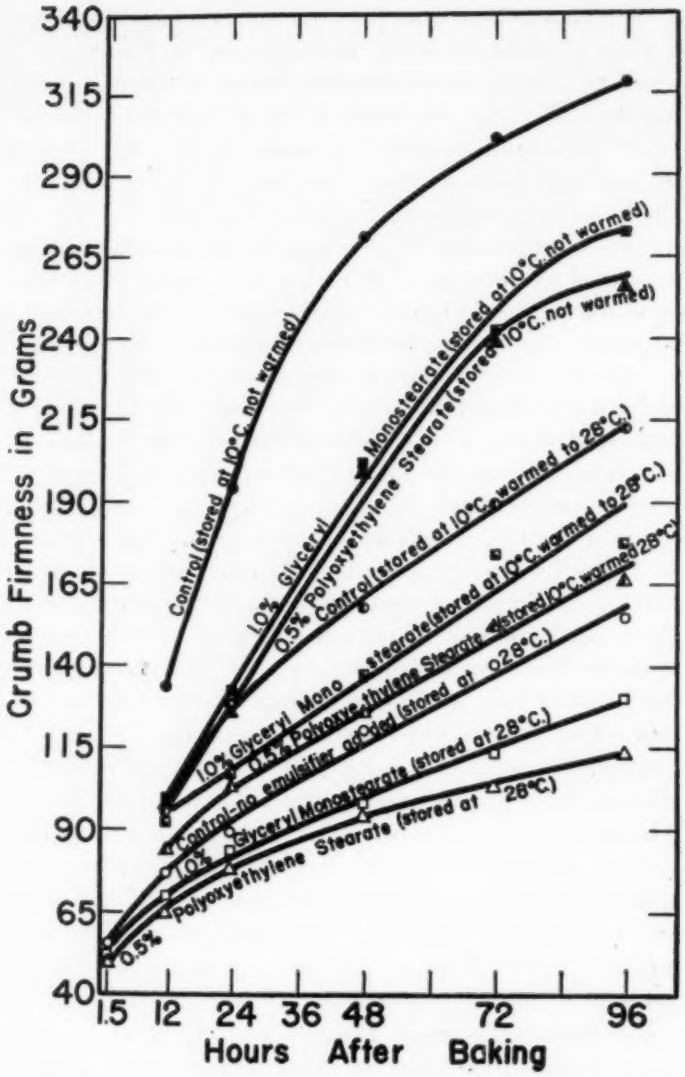

Fig. 4. Effect of emulsifiers on the degree and rate of increase in crumb firmness of bread stored at 10°C. and at room temperature (approximately 28°C.) using the Bloom Gelometer. Polyoxyethylene stearate in plastic form (100% active) and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

emulsifiers. However, bread containing an emulsifier will maintain the advantage of having a softer crumb than the control when both are stored at 10°C.

Effect of Refreshening Bread. When breads containing polyoxyethylene stearate, glyceryl monostearate, and glyceryl oleostearate and a control were heated to 60°C. in a radio-frequency field, 48 hours after baking, all were freshened to the extent that they appeared to

be oven-fresh. Compressibility tests made after the bread had cooled in 6 hours to 30°C. showed that the treatment had a softening effect on all samples (Fig. 5). Twelve hours after heating (60 hours after baking) all refreshened loaves were softer than the respective unheated loaves. However, by 48 hours after heating (96 hours after baking)

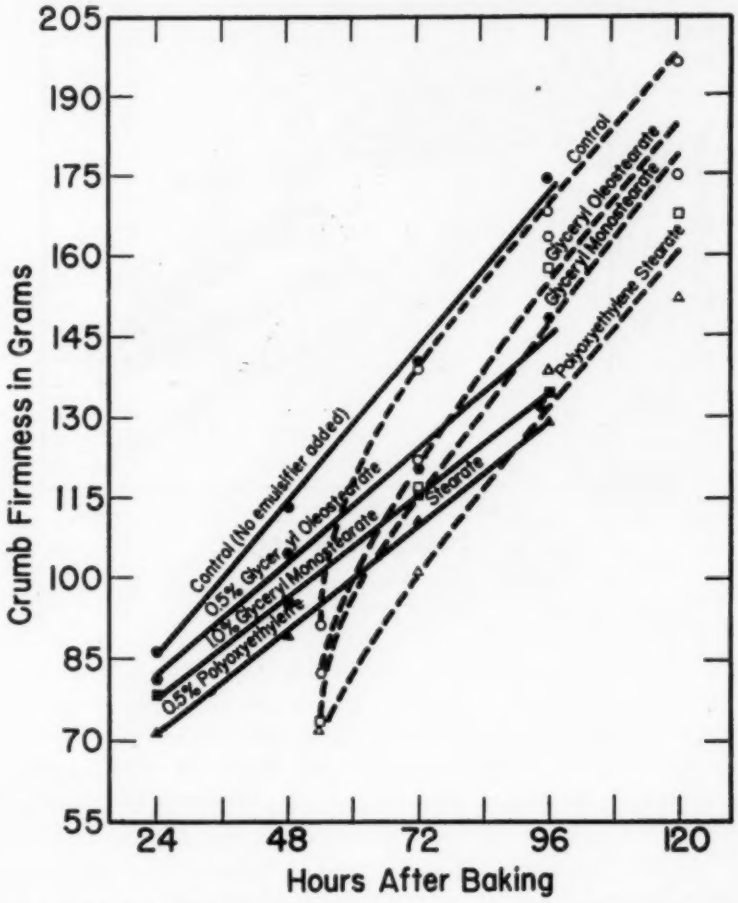

Fig. 5. Effect of emulsifiers on the degree and rate of increase in crumb firmness of bread heated to 60°C. in a radio-frequency field. Solid lines represent unheated bread; broken lines represent refreshened (heated) bread. Glyceryl oleostearate and polyoxyethylene stearate, both in plastic form (100% active), and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

all the electronically heated loaves containing emulsifiers had lost the advantage of being softer than the respective unheated loaves. From 60 hours after baking onward, unheated breads containing emulsifiers were softer than the refreshened control.

Bread wrapped in 300 MSAT cellophane lost moisture when heated to an internal temperature of 60°C. in a radio-frequency field. The

effect of refreshening on the moisture content of bread containing polyoxyethylene stearate and glyceryl monostearate and a control is shown in Fig. 6. The rate of moisture loss for all bread was increased after heating; however, the relative position of the curves remained the same.

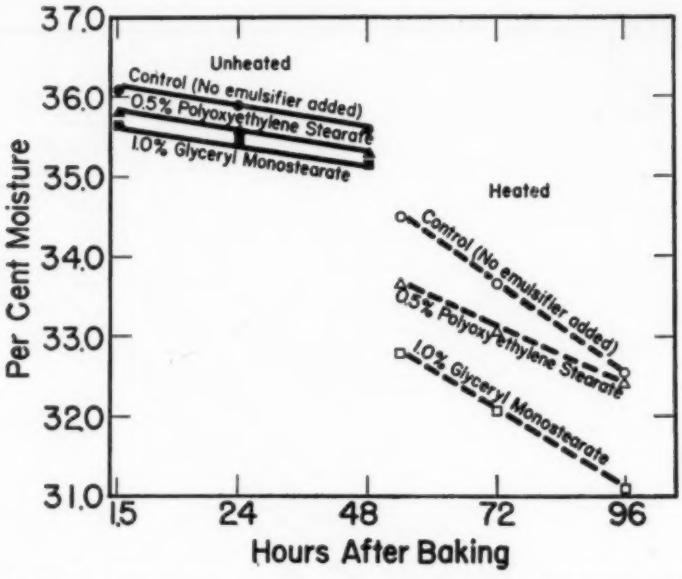

Fig. 6. Effect of emulsifiers on the rate of moisture loss of bread before and after refreshening at 60°C. in a radio-frequency field. Polyoxyethylene stearate in plastic form (100% active) and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

Effect of Emulsifiers on Moisture Retention. Moisture determinations were made on many of the bread series and showed that, with normal baking temperature and time, the addition of polyoxyethylene stearate or glyceryl monostearate did not affect the rate of moisture loss. A typical example is shown in Fig. 7. The rate of moisture loss was the same, within experimental error, for bread containing emulsifiers and the control. In some series, bread containing glyceryl monostearate had a slightly higher moisture content than either that made with polyoxyethylene stearate or the control.

Effect of Homogenization on Softness. The homogenization of various combinations of bread ingredients at 1,000 and 3,000 p.s.i. did not make bread as soft as that containing any of the five selected emulsifiers. Compressibility data showed that bread having certain ingredients homogenized had as firm a crumb as control bread during a 96-hour staling period. The homogenization of glyceryl monostearate with the ingredients usually homogenized did not improve its effectiveness in bread.

Meaning of Softness. During the staling of bread the crust becomes soft and leathery; the crumb becomes firm, harsh, and crumbly to the touch; and the bread loses moisture. Many tests have been developed and described in the literature for measuring one or more of these changes. From previous work done by the authors it is believed that compressibility testing resembling that described approaches more closely the consumer's concept of freshness than any other test. In our opinion the consumer's criterion is softness of crumb and, in general, softness and good mastication properties go together. Good

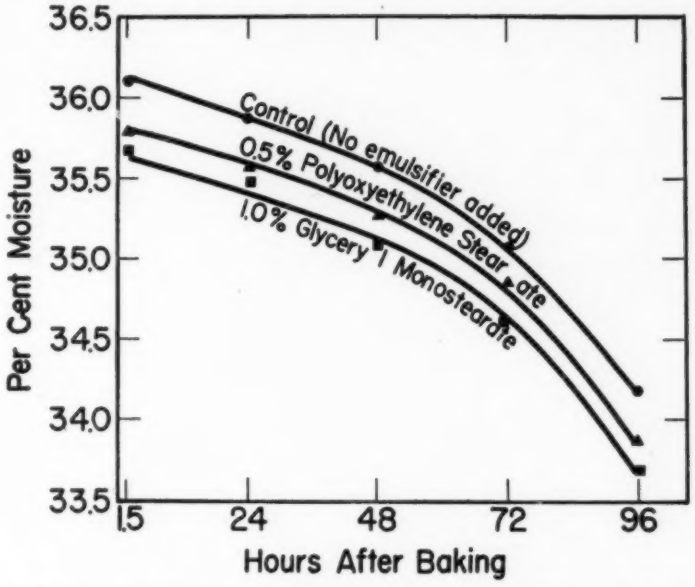

Fig. 7. Effect of emulsifiers on the rate of moisture loss of bread. Polyoxyethylene stearate in plastic form (100% active) and 3.0% of a water emulsion of glyceryl monostearate (33.3% active) were used.

correlation was noted between compressibility data obtained with the Bloom Gelometer and the "squeeze" test. Gelometer values when shown graphically reveal differences in softness that correlate with the consumer's "squeeze test." Curves showing penetrometer values do not illustrate such differences to the same extent. Although the results of this compressibility procedure do not correlate perfectly with mastication properties, the method is one of the best available at present. Crumbliness determinations were not made, but organoleptic tests showed that the five emulsifiers which were studied in detail tended to make bread more crumbly at the start. At no time, however, did this factor become undesirable, and in was felt that it was more a crumbliness that is associated with a rich formula than that which is associated with staleness.

Acknowledgments

The authors express their thanks to H. E. Wilson, F. X. Ragan, R. G. Schmidt, and J. G. Braunreuther, baking technicians, who over a period of two years performed the baking tests on which this study is based. They also thank W. J. Godkin and H. G. Beattie for helpful aid and advice in the preparation of this paper.

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DIFFERENTIAL STABILITY OF ALPHA-AMYLASE AND PROTEINASE [1]

BYRON S. MILLER and JOHN A. JOHNSON

ABSTRACT

Investigations of the differential stability of alpha-amylase and proteinase employing malted wheat flour, malted barley, and commercial fungal concentrates showed that the hydrogen-ion activity of the extracts was the most important factor influencing the stability of both enzymes.

Alpha-amylase was stable on standing for 20 hours at 5°C. over a rather narrow range of pH, depending on the enzyme source. Proteinase was stable over a wide pH range under these same conditions. The differential effect of pH on enzyme stability (5°C., 20 hours) was utilized as a means of separating alpha-amylase and proteinase in malted wheat flour and malted barley. This technic was not applicable to the fungal preparation.

Differential thermal inactivation studies at various hydrogen-ion concentrations (pH 3.0–10.5) demonstrated that the stability of both enzyme systems was markedly affected by relatively high temperatures, 50° to 60°C.

Optimum conditions for the differential inactivation of proteinase by heating at 50°C. for 30 minutes were a pH of 9.3 for malted barley, 10.2 for malted wheat flour, and 10.5 for the fungal preparation. Under these conditions complete inactivation of the proteinase could be obtained with a maximum retention of 80% of the alpha-amylase. Similar treatment employing a pH of 3.6 resulted in complete inactivation of the alpha-amylase and a maximum retention of 66% of the proteinase. Conditions for the differential inactivation of alpha-amylase and proteinase in fungal preparations were variable depending on the preparation tested.

Within wide limits, the enzyme stability did not appear to be a function of enzyme concentration. The concentration of accompanying substances, however, appeared to be a factor of prime importance bearing on stability.

The evaluation of the significance of both alpha-amylase and proteinase in breadmaking and other fermentation industries has been complicated by the lack of suitable means for separating or differentially inactivating either of these two enzyme systems. Preparations of proteinase and alpha-amylase uncontaminated with each other would be useful agents for further research. Certain accepted concepts may

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be modified or perhaps new ones evolved if experiments were performed using such enzyme systems. The development of technics whereby such preparations could be obtained easily and practicably was the primary goal of this investigation.

Research by many workers summarized by Caldwell and Adams (1) and Kneen, Sandstedt, and Hollenbeck (5) shows that alpha-amylase from various sources is more sensitive to high hydrogen-ion activities than is beta-amylase. This property serves as a means for separating the alpha and beta components. In contrast, Mounfield (9) reported that wheat proteinase is relatively stable in buffer solutions maintained as low as pH 4, but is destroyed at pH 8 at 18°C. in less than 3 days.

In an extensive study of the separation of the alpha- and beta-amylase components of malt, Kneen, Sandstedt, and Hollenbeck (5) defined the conditions of time, temperature, pH, and the presence or absence of supplementary calcium-ions necessary for the differential inactivation of either component. Maximum retention of alpha-amylase (90 to 100%) and complete inactivation of beta-amylase were attained in the presence of calcium-ion at a pH of 6.0 to 7.0 and a temperature of 70°C. Conversely, maximum retention of beta-amylase and complete inactivation of alpha-amylase were attained in the absence of calcium-ion at a pH of 3.0 and a temperature of 30°C. The influence of these conditions on the activity of proteinase was not investigated.

Hildebrand (3), Miller and Johnson (7), and Dirks and Miller (2) have reviewed the literature dealing with means of separating the proteinase from amylolytic preparations. Most early workers have relied on the specific action of safranine to remove the proteinases from amylolytic preparations. Dirks and Miller (2) investigated a number of adsorbents with respect to their ability to remove proteolytic enzymes selectively. Associated treatments including the influence of high hydroxyl-ion concentration were also investigated. From 50 to 60% of the proteolytic activity in a mold bran extract was inactivated irreversibly by the adjustment of the hydrogen-ion concentration to pH 10.0. Under these conditions little decrease in amylolytic activity occurred. Similar effects were noted for extracts of malted wheat flour and malted barley.

The present investigation was undertaken following the promising results obtained in the previous study by Dirks and Miller (2) using high hydroxyl-ion concentrations to remove the proteinase from fungal extracts, and the work of Kneen, Sandstedt, and Hollenbeck (5) concerning the stability of amylases. Included in the study were the effects of time, temperature, and hydrogen-ion concentration on the

differential stability of alpha-amylase and proteinase in various amylolytic preparations.

Materials and Methods

Three enzyme sources were investigated. These included a commercial malted wheat flour, a five-day malted barley, and a commercial fungal concentrate possessing 42.8, 81.4, and 3300 alpha-amylase units per gram, respectively. In addition, a series of malted wheat flours, malted barleys, and several fungal preparations having a wide range of enzyme activity was studied.

Enzyme solutions were prepared by a one-hour extraction (30°C.) of the original preparation. Adjustment to a pH value appropriate for maximum stability was effected as soon as practicable. The fungal concentrate was extracted in the ratio of 2 mg. per ml. of extractant. The malted wheat flour and the malted barley (freshly ground on a Wiley mill using a 1 mm. screen) were extracted in the ratio of 2 g. to 5 ml. of liquid. Solutions from which the proteinase was to be removed were extracted and diluted with 0.2% calcium chloride solution. Solutions from which the alpha-amylase was to be removed were extracted and diluted with distilled water.

All pH values were measured or adjusted by the use of glass electrode equipment. Instrument calibrations were performed using a saturated solution of potassium acid tartrate (pH 3.7) and Clark and Lubs buffer solutions. Readings over the entire range were checked against readings obtained with a second instrument.

The effects of temperatures ranging from 5° to 60°C. and of hydrogen-ion concentrations from pH 3.0 to 10.5 were investigated. In all cases care was exercised to insure uniformity of treatment and minimal loss by evaporation. The pH values of the solutions were changed arbitrarily by the addition (with rapid mechanical stirring) of either 1.0 *N* sodium hydroxide or 1.0 *N* sulfuric acid. All solutions were readjusted to 30°C. and to a stable pH after treatment and before enzyme activities were determined.

Alpha-amylase determinations were made according to the procedure of Sandstedt, Kneen, and Blish (10). Determinations of proteolytic activity were made using the Ayre-Anderson procedure as modified by Miller (6).[2]

Results and Discussion

Stability of Alpha-Amylase and Proteinase at Various pH Levels. The apparent greater proteinase activity for preparations extracted

[2] It is recognized that the method for determining proteolytic activity using hemoglobin as an auxiliary substrate is empirical and may or may not be correlated with proteolysis of other substrates.

with 0.2% calcium chloride (pH 6.0) compared with extractions made with distilled water (pH 7.6) and the previous work reported by Mounfield (8) led to a study of the stability of the enzymes in solutions of various hydrogen-ion concentrations. It has been established earlier by Dirks and Miller (2) that 0.2% calcium chloride did not influence the proteinase activity appreciably. A graphical summary of the data obtained at various pH values with extracts of malted wheat

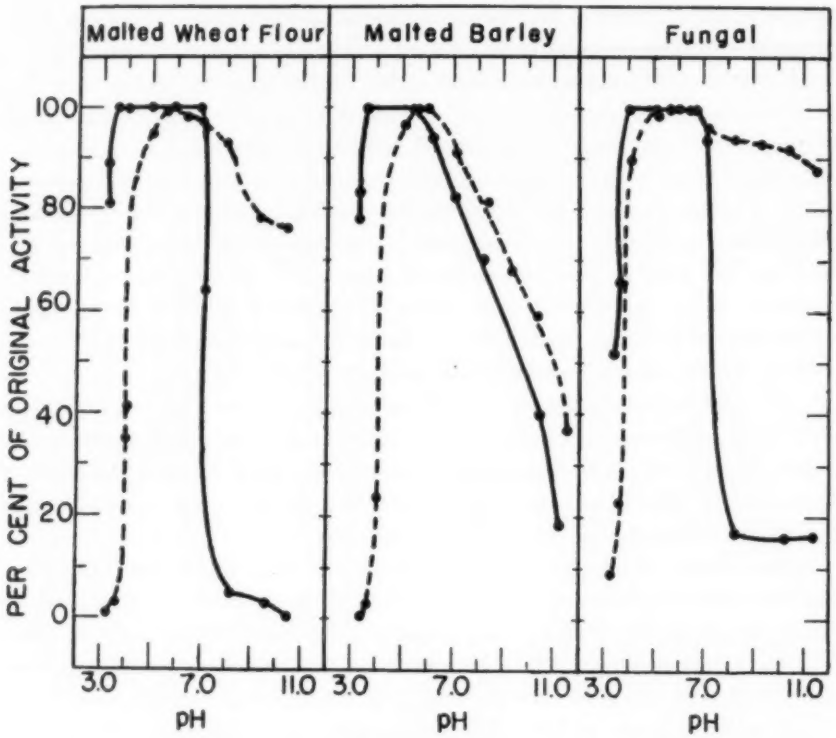

Fig. 1. Stability of alpha-amylase and proteinase in extracts of malted wheat flour, malted barley, and a fungal preparation standing at various hydrogen-ion concentrations (20 hours, 5°C). Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

flour, malted barley, and a fungal concentrate is presented in Fig. 1. All solutions stood 20 hours in a refrigerator (5°C.) at the indicated pH.

Mounfield (8) found that the storage of aqueous extracts of sprouted wheat in the dark at 18°C., in the presence of toluene, resulted in a progressive loss of proteolytic activity such that one-half of the original activity was lost in 18 days. This aging process was accompanied by a deepening of the extract color, the appearance of a precipitate, and a decrease in pH value. The inactivation was found to follow the course of a unimolecular reaction. The results obtained

in the present study indicate that this inactivation can be speeded up by the selection of an appropriate pH.

The relative positions of the activity curves for malted wheat flour and malted barley (Fig. 1) show that this technic may be applied as a means of differentially inactivating the alpha-amylase in both malted wheat flour and malted barley. This method, however, does not permit the differential inactivation of the proteinase in malted barley, although it appears to be effective for malted wheat flour.

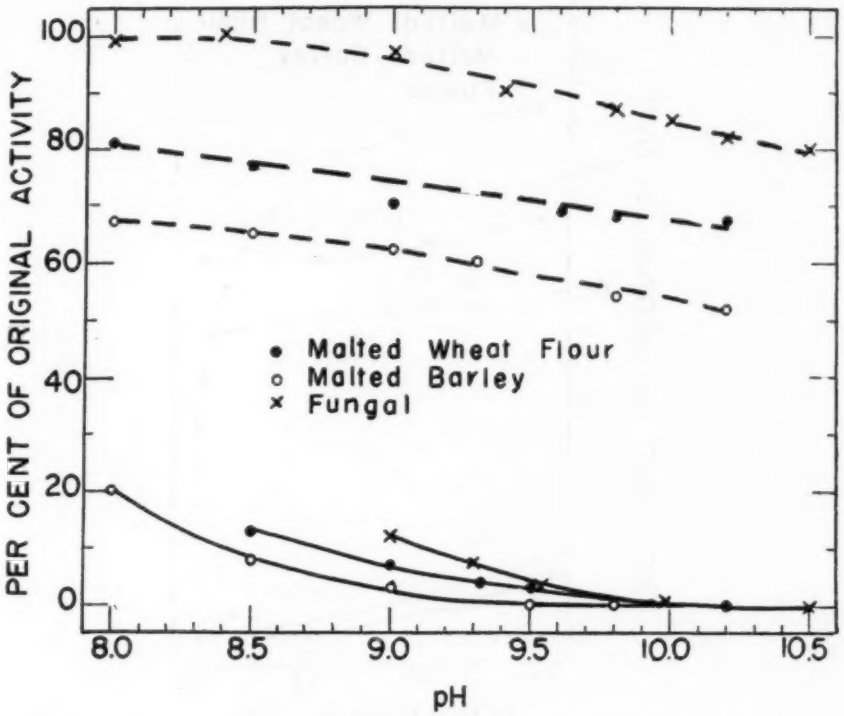

Fig. 2. Influence of high pH on the thermal inactivation (30 minutes, 50°C.) of proteinase in extracts containing alpha-amylase. Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

The close proximity of the curves representing the activities of the two enzyme systems in the fungal preparation precludes the application of this means for differentially separating either alpha-amylase or proteinase in this particular sample. Preparations produced from the growth of other organisms or under different cultural practices may show different properties.

Differential Inactivation of Proteinase and Alpha-Amylase at High pH. A study of the manner in which alpha-amylase and proteinase are influenced by hydrogen-ion concentration necessarily has involved

a consideration of the time of contact under specified conditions of temperature and hydrogen-ion concentration. The percentages of alpha-amylase and proteinase remaining after heating extracts of malted wheat flour, malted barley, and a fungal amylase preparation at various low hydrogen-ion concentrations are shown in Fig. 2. These studies were conducted under conditions of high pH (8.0 or above) and involved heat treatment at 50°C. for 30 minutes such that the major

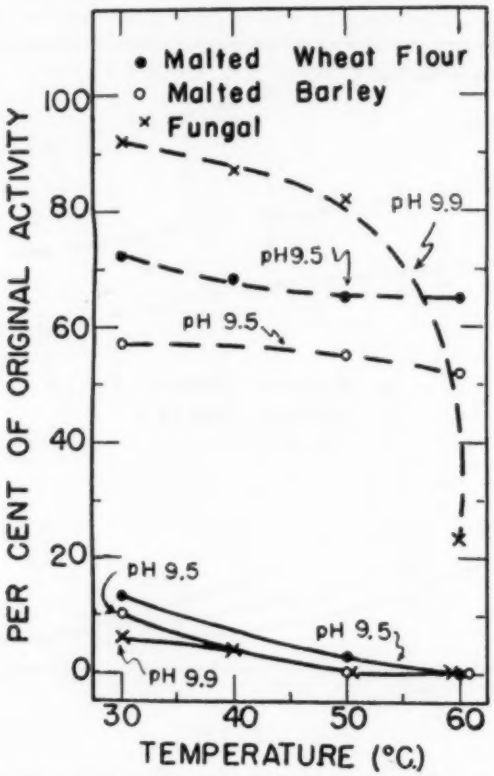

Fig. 3. Influence of temperature on the differential thermal inactivation of proteinase in extracts containing alpha-amylase. Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

influence was on the proteinase system rather than on the alpha-amylase system.

It is apparent from these data that there is little difference in the susceptibility of the proteinase from the three preparations to inactivation at high pH. The most stable proteinase system was that present in the fungal preparation which required the adjustment of the pH to 10.5 in order to destroy the proteinase completely.

Fungal alpha-amylase also was more stable than that present in the other preparations. This is surprising in view of the known thermo-

lability of fungal amylase in the pH range of 5.0 to 6.0 (4). At pH values suitable for minimum retention of proteinase, approximately 80% of the fungal alpha-amylase was retained as compared with 60 to 65% in the malted wheat flour and malted barley, respectively.

The influence of temperature on the differential inactivation of proteinase at selected high pH values is graphically illustrated in Fig. 3. Heating at temperatures ranging from 50° to 60°C. caused little change in the amount of alpha-amylase retained in malted wheat flour

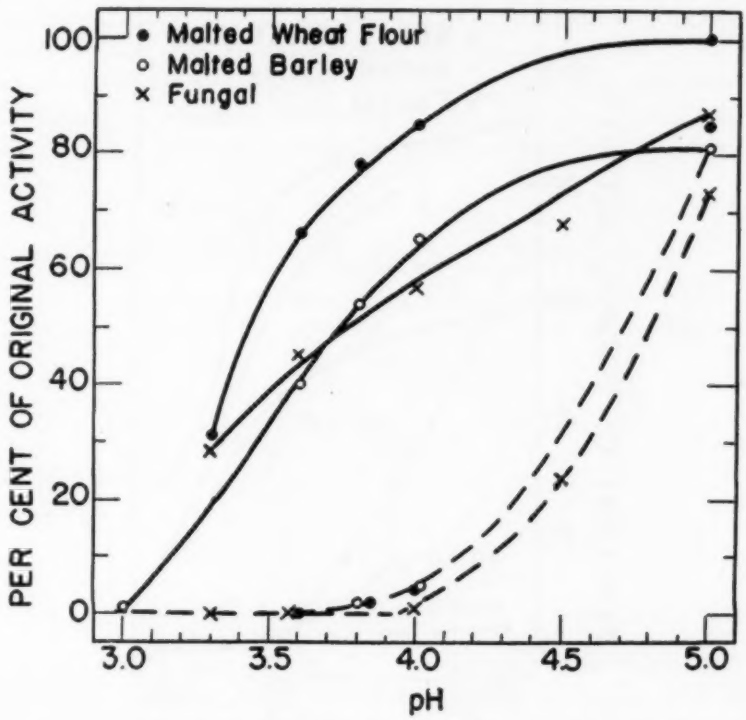

Fig. 4. Influence of low pH on the thermal inactivation (30 minutes, 50°C.) of alpha-amylase in extracts containing proteinase. Solid lines represent retention of proteinase and the dashed lines represent retention of alpha-amylase.

or malted barley extracts, but a sharp decrease in the amount of alpha-amylase retained by the fungal preparation. It was necessary to heat malted wheat flour extract at 60°C. to inactivate the last traces of proteinase. Inactivation of the proteinase in extracts of malted barley and of the fungal preparations, however, appeared to be essentially complete at 50°C.

The time of heating was not critical, although prolonged heating up to 60 minutes caused a slight decrease in the activity of both enzyme systems. An increase in heating time from 10 to 60 minutes

(50°C. and pH 9.5) resulted in a decrease in amylolytic and proteolytic activity for malted wheat flour amounting to 7 and 3%, respectively. For the fungal preparation adjusted to pH 9.9 and heated at 50°C. this decrease amounted to 5 and 0.5%.

Considerable flocculation of enzymatically inactive material occurred during the heating of the extracts at high pH (8.0–10.5). This technic might serve, therefore, as a preliminary purification step

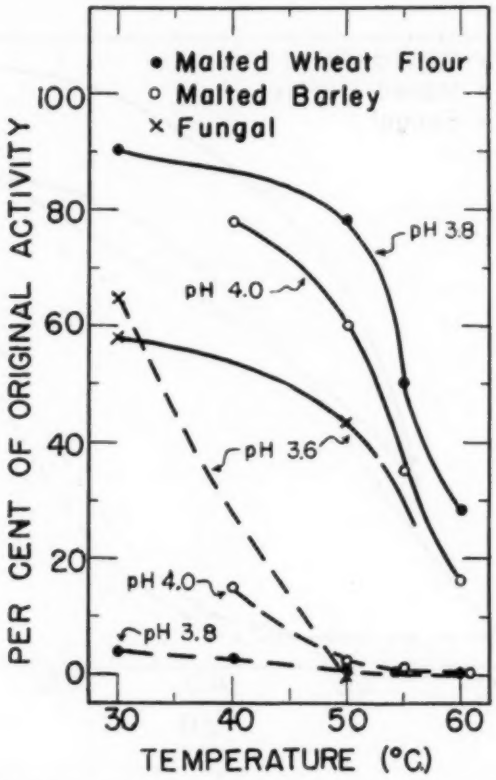

Fig. 5. Influence of temperature on the differential thermal inactivation of alpha-amylase in extracts containing proteinase. Solid lines represent retention of proteinase and dashed lines represent retention of alpha-amylase.

in the preparation of an amylase concentrate. Similarly, the heat treatment of extracts at low pH resulted in the flocculation of smaller but significant amounts of inactive material.

Differential Inactivation of Alpha-Amylase and Proteinase at Low pH. In crude aqueous extracts the alpha-amylase of malted wheat flour, malted barley, and fungal preparations was more sensitive to high hydrogen-ion activities than was the proteinase. This fact has been utilized in removing alpha-amylase from extracts containing

proteinase. Again, the manner in which this differential inactivation takes place necessarily involved a consideration of the time of contact under specified conditions of temperature and pH. The percentages of alpha-amylase and proteinase remaining in the three enzyme sources after heating the extracts (30 minutes, 50°C.) at various hydrogen-ion concentrations are shown in Fig. 4.

Heating (50°C. for 30 minutes) at a pH value of 3.6 was necessary to remove essentially all of the alpha-amylase from these preparations. Under these conditions 66% of the malted wheat flour proteinase, 39% of the malted barley proteinase, and 45% of the fungal proteinase were retained. Thus, malted wheat flour proteinase appears to be more stable to inactivation at low pH than that present in malted barley or in the fungal preparation. Heating at 50°C. for 30 minutes (pH 4.0) resulted in a substantial increase of proteinase retained by each prep-

TABLE I

OPTIMUM CONDITIONS FOR DIFFERENTIAL INACTIVATION OF ALPHA-AMYLASE IN EXTRACTS CONTAINING PROTEINASE

(Percentages of original activity remaining)

Enzyme source	Treatment			Alpha-amylase	Proteinase
	$[H^+]$	Temp.	Period		
	pH	°C.	*hrs.*	%	%
Malted wheat flour	3.6	50	0.5	0.3	66
	3.6	5	20	3.5	80
Malted barley	3.3	50	0.5	0	29
	3.8	50	0.5	2	54
	3.6	5	20	0.4	85
Fungal concentrate	3.6	50	0.5	0	45

aration. There was, however, a concomitant small increase in the amount of alpha-amylase retained, varying from 1% for the fungal preparation to 4% for both the malted barley and malted wheat flour.

The effect of heating extracts at selected pH values for 30 minutes at different temperatures is illustrated in Fig. 5. The most significant result is the sharp decrease in the percentage of proteinase retained in the extracts when these preparations were heated at temperatures varying from 50° to 60°C.

Optimum Conditions for Inactivation of Proteinase or Alpha-Amylase. The optimum conditions for maximum retention of proteinase together with complete or nearly complete inactivation of alpha-amylase are summarized in Table I. It is possible, for each enzyme preparation, to obtain proteinase completely free from alpha-amylase, although there also may be considerable loss in proteinase activity.

In some cases, a better separation of alpha-amylase and proteinase was obtained by allowing the extract to stand in the refrigerator for 20 hours after adjustment to the proper pH value. In other instances, a heat treatment after adjustment to a selected pH value may be more desirable. The method employed depends on the degree of separation desired.

A summary of the data obtained for a series of malted wheat flours and malted barleys possessing a wide range of enzyme activity is presented in Table II. It would appear from these results that the

TABLE II

INACTIVATION OF ALPHA-AMYLASE FROM A SERIES OF MALTED WHEAT FLOURS AND MALTED BARLEYS POSSESSING A WIDE RANGE OF ENZYME ACTIVITY

(Percentages of original activity remaining)

Sample no.	Alpha-amylase	Proteinase activity	Treatment			Amylase	Proteinase
			[H+]	Temp.	Time		
	units	*ml.*	*pH*	*°C.*	*hrs.*	%	%
			MALTED WHEAT FLOURS				
A	30.8	1.61	3.6	50	0.5	0.3	65
B	60.0	2.12	3.6	50	0.5	0.3	77
C	85.7	2.52	3.6	50	0.5	0.6	79
D	109.1	2.83	3.6	50	0.5	0.5	66
A	30.8	1.61	3.6	5	20	3.6	84
B	60.0	2.12	3.6	5	20	3.4	81
C	85.7	2.52	3.6	5	20	3.7	78
D	109.1	2.83	3.6	5	20	3.7	82
			MALTED BARLEYS				
1	41.0	1.73	3.6	5	20	0.5	86
2	85.7	2.70	3.6	5	20	0.3	79
3	126.3	3.20	3.6	5	20	0.4	80
4	145.5	3.31	3.6	5	20	0.2	86
5	150.0	3.69	3.6	5	20	0.4	81

degree of differential separation of the enzymes bears no relation to the enzyme activities of the original preparations. Thus, heating an extract of malted wheat flour (pH 3.6, 50°C., 30 minutes) resulted in a loss of all but 0.3 to 0.6% of the alpha-amylase and a retention of 65 to 79% of the proteinase. Employing other conditions (pH 3.6, 5°C., 20 hours), similar uniformity was obtained but greater percentages of both enzymes were retained. Treating extracts of malted barleys similarly resulted in a loss of all but 0.2 to 0.5% of the alpha-

TABLE III

OPTIMUM CONDITIONS FOR DIFFERENTIAL INACTIVATION OF PROTEINASE IN EXTRACTS CONTAINING ALPHA-AMYLASE

(Percentages of original activity remaining)

Enzyme source	Treatment			Alpha-amylase	Proteinase
	[H+]	Temp.	Time		
	pH	°C.	*hrs.*	%	%
Malted wheat flour	9.5	60	0.5	65	0
	10.2	50	0.5	67	0
	10.0	5	20	65	0.1
Malted barley	9.3	50	0.5	60	0
Fungal concentrate	10.5	50	0.5	80	0

amylase and a retention of 79 to 86% of the proteinase. No conditions were found that were optimum for the differential separation of proteinase and alpha-amylase in several fungal preparations.

Maximum retention of alpha-amylase together with nearly complete inactivation of proteinase was best obtained under the optimum

TABLE IV

INACTIVATION OF PROTEINASE FROM A SERIES OF MALTED WHEAT FLOURS AND MALTED BARLEYS POSSESSING A WIDE RANGE OF ENZYME ACTIVITY

(Percentages of original activity remaining)

Sample no.	Alpha-amylase	Proteinase activity	Treatment			Alpha-amylase remaining	Proteinase remaining
			[H+]	Temp.	Time		
	units	*ml.*	*pH*	°C.	*hrs.*	%	%
MALTED WHEAT FLOURS							
A	30.8	1.61	9.5	60	0.5	67	0
B	60.0	2.12	9.5	60	0.5	74	0
C	85.7	2.52	9.5	60	0.5	61	0
D	109.1	2.83	9.5	60	0.5	60	0
A	30.8	1.61	10.0	5	20	64	0.1
B	60.0	2.12	10.0	5	20	67	0.1
C	85.7	2.52	10.0	5	20	64	0.1
D	109.1	2.83	10.0	5	20	61	0.1
MALTED BARLEYS							
1	41.0	1.73	9.3	50	0.5	66	0.1
2	85.7	2.70	9.3	50	0.5	60	0.1
3	126.0	3.20	9.3	50	0.5	56	0.1
4	145.5	3.31	9.3	50	0.5	62	0.1
5	150.0	3.69	9.3	50	0.5	60	0.1

conditions of pH, time, and temperature recorded in Table III. A summary of the percentages for both enzymes remaining after treatment of the series of malted wheat flours and malted barleys at these optimum conditions is shown in Table IV. Differential separation of alpha-amylase from proteinase was again independent of the enzyme activities in the original samples. In all cases, less than 0.1% of the original proteinase activity was retained. The per cent of alpha-amylase retained varied from 56 to 66% for malted barleys and from 60 to 74% for the malted wheat flours.

The results obtained from a series of fungal preparations were exceedingly variable. This suggests that the type of organism used and the cultural practices involved may influence the inactivation of alpha-amylase and proteinase. The concentration of accompanying substances also may exert a marked influence on the stability of the enzyme. This was demonstrated by the results from an experiment in which a fungal preparation was diluted with an equal portion of autoclaved malted wheat flour extract, prior to subjecting the preparation to the normal thermal inactivation technic employing low pH. The per cent of alpha-amylase remaining was of the order of 65%, the same as that remaining when a malted wheat flour extract was subjected to the same treatment. The control, using the fungal preparation diluted with an equal portion of water, retained 80% of the alpha-amylase.

It is well to stress, therefore, that the complete inactivation of either alpha-amylase or proteinase cannot be assumed merely because the extract has been subjected to the prescribed conditions recorded in Tables I and III. This is especially true for fungal preparations but to a lesser degree for extracts of malted wheat flour or malted barley. An analysis for enzyme activity should always be made before and after the desired treatment.

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STUDIES ON THE ROLE OF ALPHA-AMYLASE AND PROTEINASE IN BREADMAKING

JOHN A. JOHNSON and BYRON S. MILLER [1]

ABSTRACT

Alpha-amylase and proteinase supplements prepared by differential inactivation from malted wheat flour and fungal extracts have been utilized to evaluate the role of these enzyme systems in baking using a commercial sponge-dough procedure. Three flours were studied, including commercially milled hard red spring and winter wheat flours and a composite of experimentally milled flour.

Increasing the concentration of alpha-amylase up to 20 times normal concentration increased the loaf volume. The grain and texture was improved with normal concentrations of alpha-amylase but became soft and gummy and lacking in body with higher concentrations. Alpha-amylase caused the crumb to be more compressible.

The effects of increasing the concentration of proteinase differed with the malted wheat flour and the fungal extracts and with the flour employed. Hard red spring wheat flour exhibited a marked response in loaf volume but the winter wheat flour did not respond in this manner. Higher concentrations of proteinase derived from the fungal preparation were detrimental to loaf volume, grain, and texture and decreased crumb compressibility.

Alpha-amylase is largely responsible for the decrease in dough consistency at normal concentrations of a malt supplement. The consistency of dough remained constant when the concentration of alpha-amylase was increased beyond the normal supplementation practice. The effect of the proteinase in decreasing the dough consistency continued with higher con-

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centrations of the supplement. The decrease in dough consistency may be considerable, depending upon the amount of supplement employed and the ratio of alpha-amylase to proteinase activity.

The role of amylase supplements in breadmaking has been investigated extensively (Kneen and Sandstedt, 9; Geddes, 5; and Hildebrand, 6). The attempts to separate and study the effects of each component have failed to be fully conclusive. The recent work of Miller and Johnson (15) and Dirks and Miller (2) on differential separation of alpha-amylase and proteinase in malt supplements provides materials with which the role of these enzyme components in breadmaking may be reinvestigated.

The role of alpha-amylase in increasing the sugar production, and thereby increasing gas production and improving crust color, is commonly accepted (Freeman and Ford, 3). Other roles, however, that alpha-amylase may play are not so clearly demonstrated. Controversy exists as to what component of amylase supplements is responsible for changes in dough consistency, loaf volume, grain and texture, and crumb compressibility. Sherwood and Bailey (21), employing large dosages of malted wheat flour in baking with a straight dough formula, concluded that impaired gas retention resulted from excessive proteolysis. Kozmin (11), working with flour from sprouted grain, demonstrated that the detrimental effects of these flours compared with normal flours was due to excessive splitting of the starch with consequent formation of sticky dextrins, reducing sugars, and releasing water in the crumb.

Miller and Johnson (14) demonstrated that the proteolysis might be excessive in sponge doughs. Salt employed in the straight dough inhibited about 60% of the proteolysis.

Tissue and Bailey (22), Read and Haas (18), Munz and Bailey (17), and Hildebrand and Burkert (7) have employed safranine dye as originally suggested by Marston (12) to precipitate the proteinase from the other enzyme components of malt supplements. Certain conclusions were reached by these authors concerning the role of alpha-amylase and proteinase in baking. Tissue and Bailey (22) concluded that alpha-amylase was responsible for increased loaf volume and improved grain and texture of the baked bread. Munz and Bailey (17) employed a high hydrogen-ion concentration to inactivate the alpha-amylase, and concluded that alpha-amylase was responsible for changes in dough mobility during fermentation. It is possible, however, that the proteinase enzyme may have been inactivated by the citric acid employed in lowering the pH of the extract (Mounfield, 16).

Read and Haas (18) concluded that small quantities of proteases were beneficial with "bucky" type doughs because of the mellowing

effects on the gluten. Excessive dosages of diastatic agents were detrimental because of stickiness imparted to the dough. Hildebrand and Burkert (7) indicated that increased dough mobility was due to alpha-amylase and not to the proteases. Sandstedt, Jolitz, and Blish (19) and Kneen and Sandstedt (10) believed that most of the effects of malt supplements can be explained by the action of alpha-amylase on the starch. These authors did not, however, investigate experimentally the effects of the malt proteinases. Kneen and Sandstedt (10) pointed out that the increase in loaf volume was achieved by increasing the gas retention which was modified as a result of the formation of dextrins in the dough.

Bailey (1) demonstrated that malt extracts produced a softer bread crumb but did not determine what enzyme systems were responsible for this action.

Recent methods (15) of differentially separating the alpha-amylase and proteinase of malt extracts make possible a study of the individual components. Therefore, the object of this study has been to reinvestigate the role of alpha-amylase and proteinase in breadmaking. Included in these studies are the effects of alpha-amylase and proteinase on loaf volume, grain and texture, dough consistency, and crumb compressibility.

Materials and Methods

Three samples of flour representing hard red spring wheat, hard red winter wheat, and a composite of spring and winter wheat were studied. The analytical characteristics of these flours are given in Table I. Two flours were commercially milled bakers patent, bleached

TABLE I

ANALYTICAL DATA FOR FLOURS EMPLOYED IN STUDY OF MALT SUPPLEMENTATION

Flour type	Protein[1]	Ash[1]	Water[1] absorption	Dough mixing time
	%	%	%	*min.*
Hard red spring wheat[2]	12.3	0.42	68	6.0
Hard red winter wheat[2]	11.8	.42	65	4.0
Composite of hard red spring and hard red winter wheat[3]	12.7	.44	68	5.0

[1] Expressed on 14% moisture basis.
[2] Commercially milled.
[3] Experimentally milled.

but not malted, and considered typical of the class of wheat which they represent. The third flour was a blend of experimentally milled hard red spring and winter wheats.

The two sources of amylase supplements included a commercial malted wheat flour and a commercial fungal amylase concentrate. These enzyme preparations had a potency of 42.8 and 3300 alpha-amylase units per gram, respectively.

Supplementation was accomplished by extraction of the amylase sources with water or calcium chloride, depending on requisites of the experiment. The alpha-amylase and proteinase were separated differentially by the optimum conditions described by Miller and Johnson (15). In experiments requiring only proteinase, the concentrations of the extract were adjusted to maintain the same proteolysis as would have been provided by the extract before inactivation of the alpha-amylase. Conversely, in experiments requiring only alpha-amylase, the quantity of extract was adjusted to provide the same alpha-amylase activity as would have been provided by the extract before removal of the proteinase.

Adjustment of alpha-amylase was based on the starch dextrinization procedure of Sandstedt, Kneen, and Blish (20). The concentration of extract employed in the dough was based on a relative alpha-amylase activity noted as X [2]. The proteinase activities of the extract were determined by the Ayre-Anderson method as modified by Miller (13).[3] In experiments in which either the proteinase or the alpha-amylase was removed differentially the enzymatic activity of each component was determined to ascertain actual activities of the extracts employed in baking.

All baking experiments were conducted on a pilot-plant scale employing a commercial 70% sponge-dough procedure. All malt supplementations were made in the sponge.

The formula was as follows:

Ingredient	*Percentage*	*Sponge*		*Dough*	
		lb.	*oz.*	*lb.*	*oz.*
Flour	100.0	14	—	6.0	—
Yeast	2.0	—	6.4	—	—
Arkady	0.5	—	1.6	—	—
Malt supplement	variable	variable		—	—
Sugar	5.0	—	—	1.0	—
Salt	2.0	—	—	—	6.4
Dry skim milk solids	3.0	—	—	—	9.6
Shortening (Crisco)	3.0	—	—	—	9.6
Paniplus	0.25	—	—	—	0.8
Water	variable	variable.		variable	

The sponges were mixed for 5 minutes at high speed in a horizontal Day mixer. The sponges were set at 76°–78°F. (24.4°–25.6°C.) and

[2] X concentration represents the alpha-amylase activity that would be provided in a dough by 0.25% (based on total flour in dough) of malted wheat flour having an activity of 42.8 alpha-amylase units.

[3] It is recognized that the method for measurement of proteinase activity based on its ability to hydrolyze hemoglobin is empirical. Experience has shown, however, that the results obtained by this technic are well correlated with changes in dough consistency.

fermented for 4 hours at 80°–82°F. (26.7°–27.8°C.) and 85% relative humidity.

The doughs were remixed to optimum consistency and the dough temperature controlled at 80°–82°F. The doughs were given a 40-minute floor time, scaled to 20 oz., and given 20 minutes' rest before molding. The doughs were proofed at 92°–94°F. (33.3°–34.4°C.) and 90% relative humidity for 50 minutes and baked for 30 minutes at 425°F. (218.8°C.) in a revolving reel type oven. Loaf volumes were determined while the bread was still warm, but grain and texture scores were obtained 18 hours after baking.

The effect of the enzyme components on sponge dough consistency was measured by the farinograph. A portion (530 g.) of the sponge dough after 4 hours fermentation was placed in the large bowl and consistency followed during a 15-minute mixing period. The dough consistency after 15 minutes of mixing was chosen as a measure of enzymatic changes in the dough.

TABLE II

EFFECT OF ALPHA–AMYLASE AND PROTEINASE OF ALPHA–AMYLASE SUPPLEMENTS ON BAKING CHARACTERISTICS OF HARD RED SPRING WHEAT FLOUR

Relative conc.[1]	Malted wheat flour				Fungal amylase preparation			
	Loaf volume	Grain	Texture	Compressibility	Loaf volume	Grain	Texture	Compressibility
	cc.	%	%	*g.*[3]	*cc.*	%	%	*g.*[3]
CONTROL								
0	2690	80	80	151	2690	80	80	147
1X	2769	85	85	124	2730	80	85	125
5X	2866	80	85	109	2816	90	90	111
20X	2897	60	70	132	2672	60	70	125
SUPPLEMENT MINUS PROTEINASE								
1X	2825	75	85	120	2759	75	75	105
5X	2913	85	85	116	2813	80	85	106
20X	2922	75	75	116	2894	75	75	105
SUPPLEMENT MINUS ALPHA–AMYLASE								
1X[2]	2703	75	85	132	2738	80	85	116
5X	2881	85	90	120	2622	75	80	122
20X	2956	70	80	120	2475	60	60	127

[1] X concentration is equivalent to 0.25% of malted wheat flour having an activity of 42.8 alpha-amylase units per gram.
[2] Concentration adjusted to have proteinase activity equivalent to that in the control.
[3] Grams of lead shot required to depress plunger, one inch in diameter, into crumb a distance of 4 mm. Bread stored 66 hours.

The effects of the individual enzyme components on crumb compressibility were followed using the Bloom gelometer. Compressibility of crumb was determined on three loaves from each treatment after 18 and 66 hours of storage in plastic bags sealed with drafting tape. Two readings on each of two slices, one inch thick, cut from the center of the loaf were made, making a total of 12 measurements for each treatment.

Results and Discussion

The effects of supplementation by malted wheat flour and fungal concentrate on baking characteristics and crumb compressibility are summarized in Tables II, III, and IV for hard red spring, winter, and

TABLE III

EFFECT OF ALPHA-AMYLASE AND PROTEINASE OF ALPHA-AMYLASE SUPPLEMENTS ON BAKING CHARACTERISTICS OF HARD RED WINTER WHEAT FLOUR

Relative conc.[1]	Malted wheat flour				Fungal amylase preparation			
	Loaf volume	Grain	Texture	Compressibility	Loaf volume	Grain	Texture	Compressibility
	cc.	%	%	*g.*[3]	*cc.*	%	%	*g.*[2]
CONTROL								
0	2684	80	80	132	2680	80	80	223
1X	2763	90	90	115	2771	85	85	188
5X	2896	85	70	110	2876	95	95	195
20X	3010	70	70	138	2684	60	60	194
SUPPLEMENT MINUS PROTEINASE								
1X	2756	80	85	118	2725	85	85	168
5X	2890	85	80	121	2825	85	85	172
20X	2989	70	70	115	2870	80	80	172
SUPPLEMENT MINUS ALPHA-AMYLASE								
1X[2]	2870	80	85	121	2779	85	80	184
5X	2808	80	85	124	2533	80	82	227
20X	2792	80	70	123	2584	70	75	231

[1] X concentration is equivalent to 0.25% of malted wheat flour having an activity of 42.8 alpha-amylase units per gram.
[2] Concentration adjusted to have proteinase activity equivalent to that in the control.
[3] Grams of lead shot required to depress plunger, one inch in diameter, into crumb a distance of 4 mm. Bread stored 66 hours.

a composite of spring and winter wheat flours, respectively. The series of various concentrations of the natural enzyme extracts is listed as control. The series containing only alpha-amylase is listed as supplement minus the proteinase. The series containing only pro-

teinase is listed as supplement minus the alpha-amylase. The fungal preparations were adjusted to have an alpha-amylase activity equivalent to that of malted wheat flour, but the proteinase activity of the fungal preparation was approximately 10 times that of the malted wheat flour on an equivalent alpha-amylase basis.

TABLE IV

EFFECT OF ALPHA-AMYLASE AND PROTEINASE OF ALPHA-AMYLASE SUPPLEMENTS ON BAKING CHARACTERISTICS OF A BLEND OF EXPERIMENTALLY MILLED HARD RED SPRING AND WINTER FLOURS

Relative conc.[1]	Malted wheat flour				Fungal amylase preparation			
	Loaf volume	Grain	Texture	Compressibility	Loaf volume	Grain	Texture	Compressibility
	cc.	%	%	*g.*[3]	*cc.*	%	%	*g.*[3]
CONTROL								
0	2741	90	88	145	2740	90	85	162
1X	2731	92	90	124	2730	95	95	127
5X	2878	80	82	103	2900	88	95	104
20X	2872	75	78	122	2820	60	60	109
SUPPLEMENT MINUS PROTEINASE								
1X	2744	85	80	119	2765	90	90	133
5X	2991	88	85	115	2785	85	85	135
20X	2978	73	75	122	2990	80	80	147
SUPPLEMENT MINUS ALPHA-AMYLASE								
1X[2]	2863	85	82	116	2790	88	90	129
5X	2863	95	95	121	2655	80	80	137
20X	2875	82	85	127	2550	50	50	151

[1] X concentration is equivalent to 0.25% of malted wheat flour having an activity of 42.8 alpha-amylase units per gram.
[2] Concentration adjusted to have proteinase activity equivalent to that in the control.
[3] Grams of lead shot required to depress plunger, one inch in diameter, into crumb a distance of 4 mm. Bread stored 66 hours.

Since it was recognized that crumb compressibility might be influenced by loaf volume, the compressibility expressed on a per unit basis is presented in Table V.

The Effects of Natural Enzyme Extract Which Contained Both Alpha-Amylase and Proteinase. Increasing concentrations up to 5X of both malted wheat flour and fungal extracts increased the loaf volume in all samples of flour examined except for the fungal extract which at 20X concentration did not produce a significant change in loaf volume over that of the control. The grain and texture were improved by the use

of a limited amount of either supplements. The improvements in grain and texture included smaller and more uniform cells and a softer, whiter crumb. In nearly all comparisons, optimum grain and texture were obtained with 1X concentration of both malted wheat flour and fungal extract. As the concentration of malted wheat flour extract was increased beyond 1X the grain became more open and the texture became increasingly soft. The texture became particularly

TABLE V

EFFECT OF ALPHA-AMYLASE AND PROTEINASE SUPPLEMENTATION ON CRUMB COMPRESSIBILITY PER UNIT LOAF VOLUME

Relative conc.[1]	Malted wheat flour			Fungal amylase preparation		
	Control	Minus proteinase	Minus alpha-amylase	Control	Minus proteinase	Minus alpha-amylase
	g./cc.	g./cc.	g./cc.	g./cc.	g./cc.	g./cc.
HARD RED SPRING WHEAT FLOUR						
0	0.056			0.055		
1X	.045	0.042	0.049	.046	0.038	0.042
5X	.038	.040	.042	.039	.038	.047
20X	.046	.040	.041	.047	.036	.051
HARD RED WINTER WHEAT FLOUR						
0	.049			.083		
1X	.042	.043	.042	.068	.062	.066
5X	.038	.042	.044	.068	.061	.069
20X	.046	.038	.044	.072	.060	.090
HARD RED SPRING AND WINTER WHEAT COMPOSITE FLOUR						
0	.053			.059		
1X	.045	.043	.041	.047	.048	.046
5X	.036	.038	.042	.036	.048	.052
20X	.043	.041	.044	.039	.049	.059

[1] See footnote 1 Table IV.

gummy and therefore undesirable at the highest concentrations. The crumb did not become as gummy with the fungal as with the malted wheat flour extract, but became harsh and more coarse. The detrimental effects of high concentrations of malt supplements were more pronounced with the fungal preparation than with the malted wheat flour.

The compressibility of the crumb increased up to 5X concentration, but with 20X the crumb apparently became more firm. The crumb

compressibility per unit volume (Table V) decreased up to 5X concentration but increased when 20X concentration was employed.

Effect of Supplements from Which the Proteinase Was Removed. Increasing the concentration of alpha-amylase extract caused the loaf volume to increase. In some instances of high extract concentration the loaf volume increase was greater than in the instance of the controls containing both alpha-amylase and proteinase. With every flour employing 20X concentration of fungal extract, the absence of proteinase and presence of alpha-amylase caused an increase in the loaf volume. Since sufficient sugar was provided to insure adequate gas production independent of the action of alpha-amylase, it is assumed that the alpha-amylase increased the gas retention. Further experiments showed that gas retention of the *fermenting* dough was not altered. Gas retention of the *baking* dough, however, was improved as indicated by the greater oven response when alpha-amylase was present. These results thus elaborate on the conclusions of Kneen and Sandstedt (10).

Increasing the alpha-amylase derived from either malted wheat flour or fungal concentrate up to 5X concentration improved the grain in nearly all instances. The texture also generally improved up to 5X, but with 20X concentration of alpha-amylase from malted wheat flour the crumb became gummy and moist. The gummy characteristics were not so pronounced with the fungal preparation. This difference between malted wheat flour and fungal alpha-amylase may be due to the lower temperature of thermal inactivation of the fungal alpha-amylase (Johnson and Miller, 8) with consequently less dextrin formation during the baking process. Sticky doughs were not observed at any of the concentrations of alpha-amylase employed. The effects on grain and texture of increasing the alpha-amylase in the absence of the proteinase suggest that alpha-amylase alone may not be so effective in creating improvement as when proteinase is also present in small amounts.

The effects of alpha-amylase on crumb compressibility suggest that this component of malt is mainly responsible for the increased compressibility of crumb. High concentrations of alpha-amylase, however, did not appear to be more effective than lower concentrations (Table V). No consistent difference between the effect of fungal and malted wheat flour amylases could be observed.

Effects of Supplement from Which the Alpha-Amylase Was Removed. Increasing the concentration of proteinase from malted wheat flour caused the loaf volume to increase for the spring wheat and the experimentally milled flours. The loaf volume of the hard winter wheat flour and the experimentally milled flour increased over that of the

controls but did not increase with increasing concentrations of the supplement. Improvement in grain and texture for the hard red spring and experimentally milled flour was observed up to 5X concentration of the supplement. This would suggest that with certain flours the protein may be modified to increase gas retention. These data would appear to support the conclusions of Read and Haas (18).

The effects of increasing concentrations of proteinase derived from the fungal preparation are different from those of malted wheat flour. These differences may be expected when consideration is given to the fact that the fungal preparation had 10 times more proteolytic activity than the malted wheat flour when compared on an equivalent alpha-

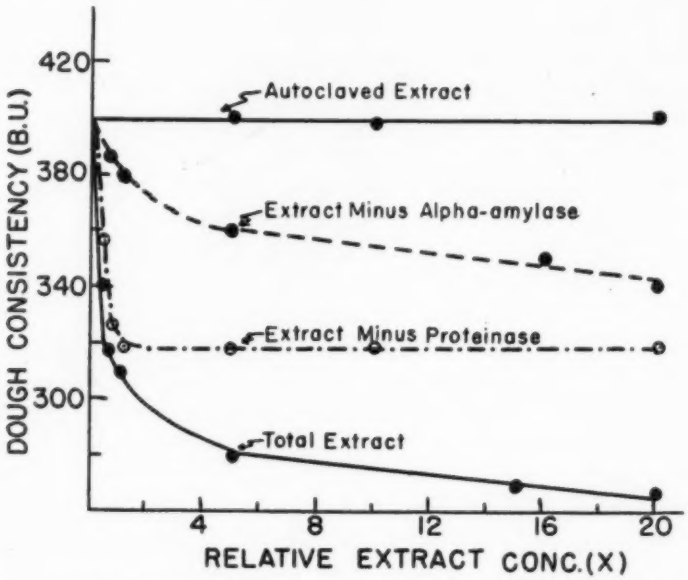

Fig. 1. Effect of normal malted wheat flour extract, extract minus alpha-amylase, extract minus proteinase, and autoclaved extract on dough consistency.

amylase basis. The amount of proteinase supplied by 1X concentration of the fungal extract was generally beneficial, but with increasing amounts the effects were detrimental. Small concentrations of proteinase caused the dough to be more mellow and extensible. Large dosages were detrimental to dough-handling properties, causing sticky and slack doughs. The data obtained from each flour supplemented with the fungal preparation consistently reveal that the presence of both alpha-amylase and proteinase up to 5X concentration improved loaf characteristics. The proteinase at the same concentration caused detrimental effects which were more pronounced in the absence of alpha-amylase. The grain and texture also show that high concentra-

tions (20X) of the proteinase supplied by the fungal extract were more detrimental than that supplied by malted wheat flour.

Small concentrations of proteinase appeared beneficial in increasing the crumb compressibility over that of the controls (Table V), but increasing amounts of fungal proteinase caused increasingly harsh and firm crumb.

Effect of the Enzyme Components of Alpha-Amylase Supplements on Dough Consistency. The effects of the enzyme components of

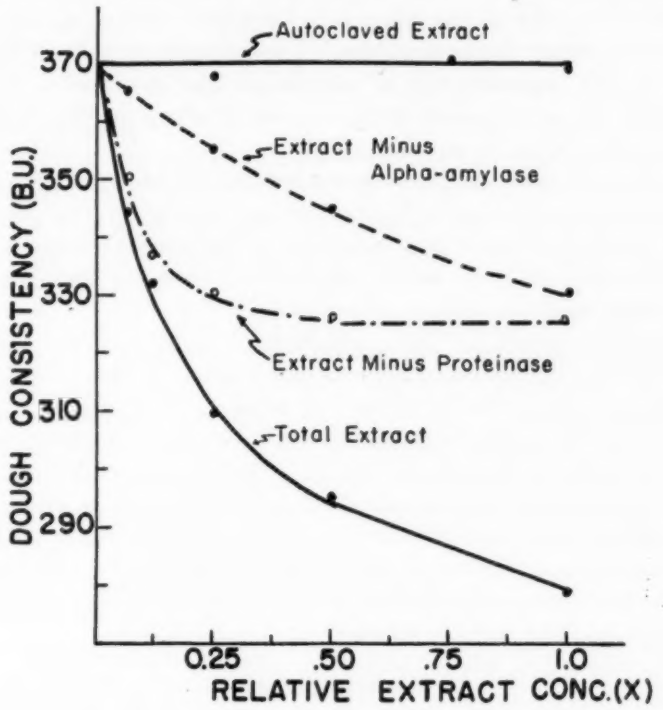

Fig. 2. Effect of normal fungal extract, extract minus alpha-amylase, extract minus proteinase, and autoclaved extract on dough consistency.

malted wheat flour and fungal preparation on dough consistency are shown in Figs. 1 and 2, respectively. That the enzymes are responsible for increased dough mobility is shown by the lack of change in dough consistency when either autoclaved extracts or extracts which had received both enzyme inactivating treatments were employed. The amount of reducing matter in the total extract as determined by iodine titration (Freilich, 4) was found to be insignificant. Sufficient yeast food was added to the sponge to counterbalance the small amount of reducing matter present.

That alpha-amylase is in part responsible for the decrease in dough consistency is evident. Alpha-amylase from both the malted wheat flour and the fungal preparation was responsible for the sharp decrease in dough consistency up to approximately 1X concentration of the enzyme. Further increase in the concentration of alpha-amylase appeared to produce no further change in dough mobility. This suggests that the quantity of starch substrate which is susceptible to attack is limited.

The doughs to which only proteinase was added also decreased in consistency, but not in the same relationship to concentration as the doughs containing alpha-amylase. Dough mobility continued to increase as the concentration of proteinase was increased. It would appear that the protein substrate was not limiting for the proteinase as was the substrate for alpha-amylase.

The effect of increasing the concentration of the total extract produced a curve, representing the combined effects of the proteinase and the alpha-amylase. The presence of both enzyme components was more effective than either component alone in decreasing the dough consistency.

The present study on the components responsible for changes in dough mobility support in part the conclusion of Munz and Bailey (17), Hildebrand and Burkert (7), and Kneen and Sandstedt (10) that alpha-amylase is largely responsible for decreased dough consistency at normal levels of supplementation. The effects of proteinase are significant, however, and cannot be discounted, particularly when an enzyme preparation is employed which has a high ratio of proteinase to alpha-amylase. The mellowing action of increased concentrations of proteinase as illustrated in Figs. 1 and 2 may, at times, be desirable, depending upon the flour employed.

Acknowledgments

Acknowledgment is given to the following companies which generously supplied the enzyme preparations used in this study: The Kansas Milling Company of Wichita, Kansas, supplied the malted wheat flour and the Rohm and Haas Company, Philadelphia, Pennsylvania, furnished the fungal concentrate, Rhozyme-S.

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THIAMINE RETENTION IN BREAD AND ROLLS BAKED TO DIFFERENT DEGREES OF BROWNNESS [1]

MARY V. ZAEHRINGER [2] and CATHERINE J. PERSONIUS [3]

ABSTRACT

Thiamine retention in yeast bread and rolls decreased as the products were baked to a darker crust color, supporting the conclusion that thiamine destruction is increased with increased exposure to heat. Thiamine retention was greater in rolls than in bread, and in clover-leaf and pan rolls than in dinner rolls. The thiamine retention in rolls was greatest in the products having the smallest surface area and the thinnest crust. The greater loss of thiamine in bread than in rolls in these experiments indicates that the length of time a product containing thiamine is exposed to heat is more important in the destruction of the vitamin than the amount of surface exposed to the heat.

The literature on yeast and quick breads reveals that the retention of thiamine is appreciably influenced by the time and temperature of baking. These two factors are so intimately related that it is impossible to study them separately in a product baked to an acceptable doneness. In general, it has been found that a high baking temperature for a short time tends to result in greater retention of thiamine than a lower temperature for a longer time (4). At any given baking temperature, as the length of time increases, the retention of thiamine decreases (11).

It has also been observed that the greatest thiamine destruction occurs in the crust of a loaf of bread, though the loss in the crumb is not insignificant (8, 9, 10, 11). The proportion of crust to crumb is greater in yeast rolls than in bread, but the baking period for the rolls is considerably shorter. A search of the literature at the time this investigation was initiated (1947) revealed no studies on thiamine retention in yeast rolls. It seemed interesting, therefore, to investigate whether the shorter baking period for rolls would compensate for the increased proportion of crust to crumb; also to compare the retentions of thiamine in yeast products baked to a dark rather than a pale crust color (both in an acceptable range).

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[2] Assistant Professor, Montana State College, Bozeman, Montana.

[3] Professor of Food and Nutrition and Head of the Department, New York State College of Home Economics, and Assistant Director of the Cornell University Agricultural Experiment Station, Ithaca, New York.

Materials and Methods

A total of 15 batches of dough was made. From each batch of dough, bread, pan rolls, clover-leaf rolls, and dinner rolls were baked. Each batch of dough was mixed from the formula given.

Formula for Water Bread and Rolls		*Per Cent of Flour*
Flour	1090.0 g.	—
Sugar	43.6 g.	4
Salt	21.8 g.	2
Fat	21.8 g.	2
Yeast	32.7 g.	3
Water	660.0 ml. (average) [4]	60.5

Ingredients. An enriched bread flour was used throughout the experiment. The amounts used were purchased as needed over a period of 8 months. This procedure was used rather than buying the entire lot at the beginning of the study since variation in the thiamine content of flour from bag to bag, even among those bought at the same time from the same mill and stored together until used, has been reported (3). The compressed yeast used in the experiments was purchased as needed in 2-oz. packages. A hydrogenated vegetable fat was used as the shortening. Tap water was used as the liquid.

A Harvard trip balance with a sensitivity of 0.1 g. was used to weigh all ingredients except the flour; the flour and the dough were weighed on a general laboratory torsion balance with a sensitivity of 0.1 g.

Mixing. A straight dough process of mixing was used. The dough was mixed in a Model H Kitchen-Aid mixer; the capacity of the mixing bowl was approximately 4 qt.

Fermentation. The dough was fermented for 75 minutes before molding. Fermentation and proofing of the doughs were carried out in a thermostatically controlled Freas Electric Oven which had been modified for use as a proofing oven and was maintained at a temperature of 30°–32°C. (81°–86°F.) and a humidity of 70% or over.

Molding. For the *bread* a 525-g. portion of dough was weighed and shaped into a loaf following the procedure of the American Association of Cereal Chemists (1). This yielded a loaf of bread weighing approximately 1 lb.

The bread was placed in dull tinned pans of standard shape, the inside top dimensions being 11 cm. × 22 cm. (8½ in. × 4¼ in.), the bottom dimensions, 9 cm. × 20 cm. (8 in. × 3¾ in.) and the depth, 8.4 cm. (3¼ in.).

For the *pan rolls* a 525-g. portion of dough was weighed and formed into a ball. This was manipulated in the same manner as the bread

[4] The amount of water used varied with the flour absorption; the range was from 630 to 686 ml.

except that instead of forming a square, this portion of the dough was formed into an oblong approximately 5 by 9 inches. It was cut into two approximately equal portions. Each piece was then rolled under the open hands to form a roll approximately 1 inch in diameter and 16 inches in length. Each piece was cut into eight approximately equal portions. Each portion was then rolled into a small ball. The 16 balls of dough were placed side by side in a lightly greased baking pan. This yielded a pan of rolls weighing approximately 1 lb.

For the *clover-leaf rolls* a 262-g. portion of dough was weighed, formed into a ball, and manipulated in essentially the same manner as for the pan rolls, except that the oblong approximated 3 by 8 inches. This was cut into two approximately equal portions and each piece was rolled under the open hands to form a roll about 12 inches in length and ¾ inch in diameter. Each roll was then cut into 12 approximately equal portions which were then rolled into smooth balls. Groups of three of these balls were placed into lightly greased muffin pan cups. This yielded eight rolls weighing approximately ½ lb.[5]

For the *dinner rolls* a 262-g. portion of dough was weighed and manipulated in the same manner as for the clover-leaf rolls up to and including dividing the oblong piece of dough. Each piece of dough was then rolled as before to form a roll about 1 by 8 inches. These were cut into four approximately equal portions which were rolled into smooth balls. Four balls were placed in each pan 1.5 cm. (⅝ in.) from the side of the pan and with 3.2 cm. (1¼ in.) of space between the balls of dough. This was done to prevent the dough from coming into contact with either the sides of the pan or another ball of dough during proofing. This yielded eight rolls weighing approximately ½ lb.[5]

The pan and dinner rolls were placed in dull tinned pans whose inside top dimensions were 18.6 cm. × 18.6 cm. (6⅝ in. × 6⅝ in.); the inside depth was 3.7 cm. (1½ in.). The clover-leaf rolls were placed in dull tinned muffin pans, the over-all dimensions of which were 26.7 cm. × 13.8 cm. (10½ in. × 5½ in.). The top and bottom radii of the cups were respectively 5.3 cm. (2¼ in.) and 4.5 cm. (1¾ in.); the inside depth was 2.5 cm. (1 in.).

Proofing. All products were placed in the proofing oven as soon as molded. The bread was always molded first; the order of molding the variously shaped rolls was rotated. The products were proofed 1 hour after the last pan was placed in the proofing oven. It took 45 minutes to prepare all of the products for the oven; consequently the

[5] The limitation of baking facilities made it expedient to use a smaller portion of dough for the clover-leaf and dinner rolls than for the loaf of bread and the pan rolls. Since the variation in thiamine content among the individual clover-leaf rolls and among the dinner rolls had been found to be within the experimental error of the method, this procedure should introduce no significant error.

breads were proofed approximately 1¾ hours, and the rolls for varying shorter times. Brooks (4) found that varying the length of time of proofing had no significant effect on thiamine in yeast breads.

Baking. The bread and rolls from a batch of dough were all baked at one time at a temperature of 204.4°C. (400°F.) in a Despatch Electric Rotary Oven, No. 150-R, which maintained the oven temperature within ±2°C. at the temperature used in the study. Five batches were baked to a pale brown crust color, five to a medium brown color, and five to a dark brown color. The length of the baking period varied as follows:

Series	*Color of Product*	*Baking Time*
I Bread	Pale[6]	30 min.
Rolls	Pale	15 min.
II Bread	Medium Brown[6]	40 min.
Rolls	Medium Brown	20 min.
III Bread	Dark Brown[6]	50 min.
Rolls	Dark Brown	25 min.

Cooling. After the products were taken from the oven, they were removed from the pans and placed on wire racks. They were allowed to cool, uncovered, at room temperature for about an hour.

Sampling. All samples were taken in duplicate. A torsion balance with a sensitivity of 0.004 g. was used for weighing the samples for thiamine and moisture determinations.

After a batch of dough was mixed, a small portion was removed to be used for thiamine, pH, and moisture determinations.[7] For the thiamine determinations, samples of approximately 5 to 6 g. were weighed into 100-ml. glass beakers and covered with watch glasses. Five milliliter beakers were filled with dough for the pH determinations. Seven to eight gram samples for moisture determinations were weighed into aluminum dry-weight pans and tightly covered.

The bread and the pan rolls were weighed and then quartered, the bread according to the method of Brooks (4), and the pan rolls as illustrated in Fig. 1. One quarter of each, to be used for thiamine and moisture determinations, was weighed, sliced, and air-dried. The dried samples were ground and aliquots were taken according to the methods of the American Association of Cereal Chemists (1). A second quarter was used for pH determinations and for subjective judging.

The clover-leaf rolls and the dinner rolls were weighed and individ-

[6] In each series, the crust color of the bread was somewhat darker than that of the rolls, due to the longer baking period necessary to bake the bread to an acceptable doneness.

[7] Preliminary work by Brooks (4) showed that samples taken from any portion of the dough gave results comparable with composite samples made up of portions taken from many different parts of the same dough.

uals[8] chosen for thiamine and moisture determinations according to the diagram in Fig. 1. The selected rolls were sliced, air-dried, and sampled in the same manner as the bread. The remaining rolls were used for pH determinations and subjective judging.

Moisture Determinations. Moisture determinations on both dough and baked products were carried out according to the methods of the American Association of Cereal Chemists (1).

pH Determinations. A Beckman glass-electrode pH meter, laboratory model, was used for all pH determinations. The pH determinations on the dough were made by introducing the electrodes directly into the dough sample. In testing the crumb of fresh bread and rolls, a piece approximately ½ × 1½ inches from the soft center

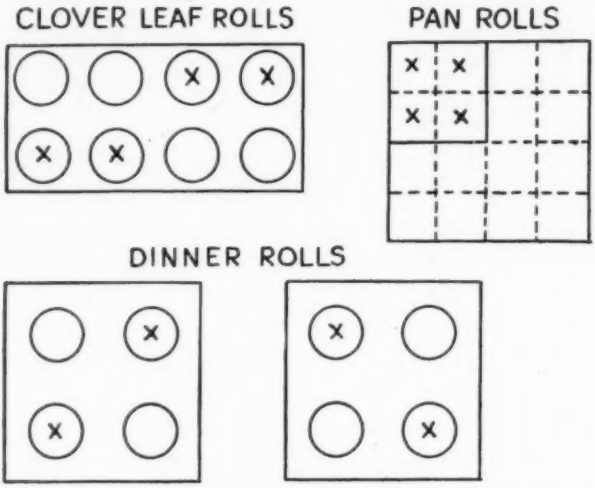

Fig. 1. Design for selecting samples of pan, clover-leaf, and dinner rolls for thiamine, pH, and moisture determinations and for subjective judging. X = rolls chosen for thiamine determinations.

was wrapped around the electrodes and held in a 5-ml. beaker while the determination was made.

Thiamine Determinations. Thiamine determinations were made on the dough and on the air-dried samples of the baked products by a procedure essentially that of Conner and Straub (5).

Expression of Results. The thiamine content of the bread and of the rolls was calculated as micrograms per gram of fresh baked product; and the percentage thiamine retention, on the basis of the total weight and total thiamine content of the products before and after baking. These methods do not require the use of dry weight data, thus eliminating any experimental errors inherent in these determina-

[8] Preliminary investigation showed that there was little variation in the thiamine content of the four quarters of a pan of pan rolls and that the thiamine content of the individual rolls from one batch of clover-leaf rolls or of dinner rolls did not vary more than 10%.

tions. They also take into account the differences in moisture content between the raw and baked product and so show the actual change in thiamine content during baking. The use of the total change method in expressing thiamine retention values was recommended by Cooperman and Elvehjem (6). This method was used by workers of the Bureau of Human Nutrition and Home Economics in reporting studies on yeast breads (12).

Results and Discussion

Dough. For the 15 batches of dough the average moisture content was 45% and the average pH 5.7. The average thiamine content of the moist doughs was found to be 2.8 μg. per gram, which is within the range generally reported in the literature.

TABLE I

AVERAGE WEIGHT LOSS IN YEAST BREAD AND ROLLS BAKED TO DIFFERENT DEGREES OF BROWNNESS [1]

Crust color	Bread %	Pan rolls %	Clover-leaf rolls %	Dinner rolls %
Pale	11.35±0.30[2]	8.46±0.10	15.57±0.47	16.11±0.22
Medium	13.90±0.29	10.36±0.16	18.17±0.77	19.39±0.86
Dark	16.95±0.27	12.23±0.47	22.14±0.92	24.43±0.62

[1] Based on the weight of the raw, molded dough.
[2] Standard error of the mean.

Bread and Rolls. All of the breads and rolls were rated "acceptable" on judging. In all cases the weight of the baked products decreased with the length of the baking period; weight loss was also influenced by the shape and size of the products (see Table I), the greatest loss occurring in the individually baked rolls. The pH of the

TABLE II

THIAMINE RETENTION IN YEAST BREAD AND ROLLS BAKED TO DIFFERENT DEGREES OF BROWNNESS DETERMINED BY ASSAYING THE TOTAL PRODUCT

Crust color	Bread %	Dinner rolls %	Clover-leaf rolls %	Pan rolls %
Pale	83.1±1.9[1]	89.5±1.1	93.0±1.5	92.6±0.7
Medium	80.4±2.1	84.5±1.2	89.8±2.0	91.5±2.1
Dark	73.8±1.5	77.5±0.6	84.0±1.0	87.8±0.8

[1] Standard error of the mean.

crumb of the baked products was not affected by any of the variables studied. The average per cent of thiamine retained in the breads and rolls baked to different crust colors, based on the total weight [9] of the dough, is presented in Table II and in Fig. 2. The average

[9] The per cent thiamine retention varies slightly with the method of calculation. The one used is considered the most reliable.

thiamine content, expressed as micrograms per gram of the fresh baked product, is reported in Table III.

Thiamine Retention. As would be predicted, in all products the retention of thiamine decreased as the crust color deepened owing to the increase in the baking time. The differences in thiamine destruction as a result of baking from a pale to a medium crust color, and from a pale or medium to a dark crust color, are significant at odds of 99:1. The percentage loss of thiamine was greater as a result of baking from a medium to a dark crust color than as a result of baking from a pale

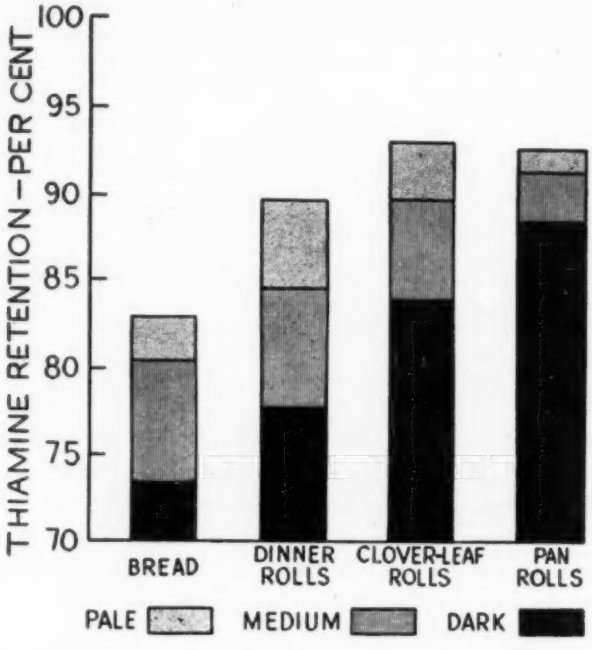

Fig. 2. Thiamine retention in yeast bread and rolls determined by assaying the total product.

to a medium crust color, indicating that thiamine destruction occurred more rapidly as the baking time was increased.

The loss of thiamine was also influenced by the shape and size of the products. In each series the retention of thiamine was greater in rolls than in bread. This finding is in general agreement with the results of studies on the thiamine retention in bread and rolls reported recently by the Bureau of Human Nutrition and Home Economics (12). They obtained retentions of 69.4 to 70.3% and 65.9% for rolls and bread respectively. Their absolute values are, however, considerably lower than those reported in this study. Since formula, baking times, and baking temperatures were different in the two studies, it is

difficult to compare the results. Comparison is further complicated by the lack of information on the shape and size of the rolls in the study by the Bureau.

Among the rolls prepared in this study the retention of thiamine was least in the dinner rolls. The vitamin retention was similar in the clover-leaf and pan rolls, although, with the exception of products baked to a pale crust color, the greatest average retention was always observed in the pan rolls. By analysis of variance the difference in thiamine retention is found to be highly significant when comparing bread and rolls, and also when comparing dinner rolls with pan and clover-leaf rolls. The difference in thiamine retention between pan and clover-leaf rolls is not significant.

Thiamine destruction has been reported as directly related to the amount of crust in baked products (2, 8, 9, 10, 11). The results of this study confirm this view. In rolls the proportion of crust to crumb varies with the surface area exposed to heat and with the thickness of the

TABLE III

AVERAGE THIAMINE CONTENT OF FRESH YEAST BREAD AND ROLLS BAKED TO DIFFERENT DEGREES OF BROWNNESS

Crust color	Bread μg./g.	Pan rolls μg./g.	Dinner rolls μg./g.	Clover-leaf rolls μg./g.
Pale	2.67±0.08[1]	2.87±0.04	3.03±0.06	3.13±0.05
Medium	2.57±0.08	2.81±0.08	2.89±0.12	3.02±0.10
Dark	2.45±0.04	2.76±0.04	2.83±0.03	2.98±0.04

[1] Standard error of the mean.

crust, both factors influenced by the type of roll. The pan rolls, in which the thiamine retention was high, had the smallest proportion of crust to crumb; the dinner rolls, in which the thiamine retention was lowest, had the greatest proportion of crust. Since the surface area exposed to heat was similar in clover-leaf and dinner rolls, it might be expected that the thiamine retention would be similar. It is believed that the greater retention in the clover-leaf rolls can be explained by the difference in the character of the crust; in the clover-leaf rolls, the crust in contact with the baking pan was thinner than that exposed to the direct heat.

Although the surface area exposed to heat would be less in a loaf of bread than in an equivalent amount of rolls, the crust of the bread was in every series rated by the judges as thicker than that of the rolls. This could account for the relatively large loss of thiamine in bread as compared with rolls. In the toasting of bread, it has been observed that the destruction of thiamine is due to the penetration of heat into the slice of bread as well as to the length of time of the toasting process

(7). Apparently the shorter exposure to heat required in baking yeast rolls compensates for the increased surface area of the rolls compared with a loaf of bread.

Thiamine Content. From Table III it will be observed that the thiamine content per gram of the various products decreased as the crust color deepened; also that the amount of thiamine was greater in rolls than in bread. Among the different types of rolls the highest content was found in the clover-leaf rolls. The higher content in dinner rolls than in pan rolls is undoubtedly a reflection of the lower moisture content of the dinner rolls. Although these differences in thiamine are small they are found by analysis of variance to be significant, indicating that the treatments accorded the samples were responsible for the differences observed.

While the findings of this study are of theoretical interest, their practical significance in the average diet is limited. Even using the extreme values, only about 10% more of the National Research Council daily recommended allowance of thiamine for a moderately active man would be furnished by six clover-leaf rolls baked to a pale brown color than by six slices of bread, made from an equal amount of dough, baked to a dark brown crust color, i.e. 36.5% of this allowance versus 25.6%.

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LIPOLYSIS IN DOUGHS MADE FROM VARIOUS CEREAL FLOURS [1]

T. WIDHE [2] and T. ONSELIUS [2]

ABSTRACT

The relative lipase activity of cereals and factors influencing lipolysis were investigated by following the increase in fat acidity of doughs containing the cereal flour under study, margarine, sucrose, and water. Flours milled from oat groats exhibited a much higher lipase activity than those made from wheat, rye, and barley. Germination of rye and oats for four days at 20° to 25°C. did not increase their lipase activity under the experimental conditions employed.

Mixtures of wheat flour and of oat flour commercially milled from oat groats prepared by the usual drying or roasting procedure prior to dehulling gave doughs which developed a soapy taste when their fat content was high. In contrast, this unpleasant phenomenon did not occur when the oat flour was milled from oat groats prepared by a commercial wet-dehulling procedure which involved washing, heat conditioning, and removal of the hulls in a centrifugal hulling machine. Laboratory experiments showed that the thermal inactivation of oat lipase is much greater when the heat treatment is carried out at 20% than at 12% moisture. That the development of a soapy taste in doughs containing certain oat flours is due to the high lipase activity of these flours, rather than to the nature of the shortening used in the baking formula, is further substantiated by the fact that several commercial fats were all strongly hydrolyzed in doughs containing unheated oat flour.

The shortage of wheat in Sweden during and since World War II made it necessary to mill mixtures of wheat with other cereals, rye, barley, and oats, to obtain sufficient flour. The addition of these cereals to wheat had a deleterious effect upon the strength and color of the flour. In addition, the flour produced by certain mills from mixtures which contained oats gave a very unpleasant soaplike taste to baked products made from doughs with a high fat content. This bad taste was most pronounced when the dough was kept for a long time, as is common in baking certain types of cakes. The soapy taste would appear to be the result of lipolytic action in the dough and the present investigation was undertaken to elucidate its cause.

The limited knowledge of the role of lipases in milling and baking has recently been reviewed by Sullivan (5). The occurrence of lipases in plants was first described in 1871 by Müntz (3), and several years

[1] Manuscript received October 4, 1948.
[2] Kvarnaktiebolaget Tre Kronor, Stockholm, Sweden.

later Fokin (2) found wide variations in the lipase content of different oil seeds. Sullivan and Howe (6) investigated the lipases of wheat, their distribution in different wheat products, and the effect of germination on lipase activity. Bamann and Ulmann (1) investigated the lipase content and pH optimum for a number of plants and plant tissues; they found the relative lipase content of oats to be 5, barley 4, and wheat very little. Secchi (4) studied the lipase activity of different wheat varieties, the temperature and pH optima for lipase, and the role of lipolysis in macaroni doughs. He found that heating the doughs at 60°C. for 20 minutes destroyed all the lipase and that the lipase activity was greatly diminished when the flour or semolina used in the doughs was rancid.

In the present study the following factors were investigated: (1) the relative lipase activities of flours milled from wheat, rye, barley, oats, and corn; (2) the influence of germination of rye and oats in the lipase activity of flours milled from them; (3) the influence of heat treatment of oats on the lipase activity of oat flour; and (4) the relative rate of lipolysis of different fats by oat flour.

Lipase Activity of Different Cereals

The relative lipase activity of flours prepared from different cereals was studied in doughs prepared by mixing thoroughly 20 g. of the particular flour under study, 10 g. of bakers' margarine, 10 g. of sucrose, and 2 g. of water. Dough samples weighing 2 g. were placed in wide test tubes which were inserted in a thermostat maintained at 20°C.[3] At appropriate intervals of time, 40 ml. of diethyl ether were stirred into the dough to extract the fatty acids and stop the reaction.[4] After filtration, 20 ml. of 96% ethanol were added and the solution was titrated with 0.05 *N* sodium hydroxide employing phenolphthalein as the indicator.

The results recorded in Table I show that the increase in fat acidity of the doughs made with wheat, rye, barley, and corn flour was very small but was rather high with oat flour. After 24 hours the doughs made from oat flour had a very strong soaplike smell and taste.

Effect of Germination on Lipolysis Produced by Rye and Oats

Rye and oat grains were soaked in water overnight and germinated between sheets of wet filter paper at 20° to 25°C. for four days; at the

[3] The buffer capacity of the doughs prevented any appreciable change in pH during lipolysis. For example, the pH of wheat flour dough after digestion for 0.25 hour was 5.80 and only decreased to 5.71 after 57 hours digestion; the corresponding pH values for oat flour dough were 5.60 and 5.45.

[4] That diethyl ether effectively extracted the fatty acids was shown by determining the acidity of the extract and of the flour-sugar residue after various intervals of digestion; while the acidity of the ether extract increased with time, that of the residue remained low and virtually constant. As constant values were obtained when aliquots of the same ether extract were titrated after standing for various periods of time, lipolysis was completely inhibited by the diethyl ether.

TABLE I

RELATIVE LIPASE ACTIVITY OF CEREAL FLOURS[1]
(Activity determined in doughs at 20°C.)

Flour	Fat acidity[2]					
	Digestion time in days					
	0	1	2	3	4	5
Wheat	0.2	0.2	—	0.3	0.3	—
Rye	0.3	0.3	0.3	0.5	—	0.5
Barley	0.5	0.6	0.4	0.5	—	0.5
Oats (dehulled)	0.5	5.0	—	8.7	9.0	—
Corn	0.6	0.5	0.4	0.5	—	0.5

[1] Approximate extractions for the flours prepared from the different cereals were: wheat, 60%; rye, 50%; barley, 60–65%; corn, 60%; and oats (dehulled) 35–40%.
[2] Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.

end of this time the sprouts had grown to at least the length of the grain. The sprouted grain was dried at room temperature, milled into flour, and the lipase activity determined in doughs prepared in the manner described in the previous section but maintained at 37°C.

The results in Table II indicate that germination did not increase the lipase activity of either oats or rye. This is very surprising as Sullivan and Howe (6) found that when wheat was germinated, the lipolytic activity increased for triglycerides of higher fatty acids but not for those of the lower fatty acids.

Influence of Heat Treatment of Oats on Lipase Activity of Oat Flour

Doughs made with oat flours from some mills developed a soapy taste while those from other mills did not exhibit this characteristic.

TABLE II

EFFECT OF GERMINATION ON LIPASE ACTIVITY OF OATS AND RYE
(Activity determined in doughs at 37°C.)

Nature of sample	Fat acidity[1]				
	Digestion time in hours				
	0.25	1	3	5	24
Oats, control	1.8	2.5	4.0	5.7	11.8
Oats, germinated	1.4	1.8	3.5	5.1	11.1
Rye, control	0.3	0.4	0.3	0.4	0.5
Rye, germinated	0.3	0.6	0.4	0.5	0.7

[1] Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.

There was reason to believe that this difference in behavior depended on the fact that the oats had been treated differently in preparing them for milling. Comparison of the increase in fat acidity of doughs made with oat flour supplied by three mills, given in Table III, shows that the flour supplied by the TK mill was much the lowest in lipase activity and that the difference could be readily detected in doughs containing only 10% of the respective oat flours. The lipase activity of oat flours from the same mill may vary rather widely, and it seemed that this might possibly be due to varietal differences. However, Swedish white and black oats, Danish white oats, and American white oats all

TABLE III

RELATIVE LIPASE ACTIVITY OF OAT FLOURS SUPPLIED BY DIFFERENT MILLS
(Activity determined in doughs at 20°C.)

Mill	Fat acidity[1]				
	Digestion time in days				
	0	1	2	3	6
Doughs made with oat flour					
TK	0.3	0.4	0.3	0.4	0.7
SV	0.9	2.7	4.0	4.9	7.4
ST	0.7	2.0	3.1	—	4.0
Doughs made with 90% of wheat flour and 10% of oat flour					
TK	0.3	0.3	0.3	0.3	0.3
SV	0.4	0.6	0.8	1.0	1.6
ST	—	1.1	—	2.0	—

[1] Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.

had practically the same lipase activity, and it is therefore more likely that the variations in lipase activity between and within mills are due to differences in the moisture content of the oats during heat treatment and in the temperature and time of heat treatment. The usual system for dehulling oats is first to subject them to a drying or slow roasting process to reduce the moisture content to 5–7% (original moisture 12–18%) after which they are dehulled between stones. In the Tre Konor mill a wet dehulling system is employed in which the oats are washed, heat-conditioned at a moisture content of 25–30%, and the hulls removed in a centrifugal hulling machine. The oat groats are then dried for 7 hours at 70°C., which lowers their moisture content to

TABLE IV

EFFECT OF HEAT TREATMENT OF OATS ON LIPASE ACTIVITY OF OAT FLOUR
(Activity determined in doughs at 37°C.)

Treatment	Fat acidity[1]				
	Digestion time in hours				
	0.25	1	3	5	24
Control, unheated	1.8	2.5	4.0	5.7	11.8
Heated at 12% moisture	1.0	1.6	2.9	3.5	9.4
Heated at 20% moisture	0.5	0.5	0.6	0.9	1.6

[1] Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.

8–9%. It seems probable that this method of dehulling will result in a greater destruction of lipases than the customary method.

To test the effect of moisture content on thermal inactivation of lipase, subsamples from one lot of oats were conditioned to 12% and 20% moisture and heat-treated in closed containers at 70°C. for 12 hours. The lipase activity of oat flour prepared from the samples was determined in doughs at 37°C. in the manner already described. The results in Table IV show that the thermal inactivation of lipase is greater when the heat treatment is carried out at 20% instead of 12% moisture. They support the opinion of the authors that the wet-hulling of oats is superior to the customary dry-hulling procedure when they are to be used to produce oat flour for mixing with wheat flour.

TABLE V

RATE OF LIPOLYSIS OF DIFFERENT COMMERCIAL FATS BY OAT FLOUR
(Activity determined in doughs at 37°C.)

Nature of fat	Fat acidity[1]				
	Digestion time in hours				
	0.25	1	3	5	24
Butter	0.9	2.2	2.5	4.9	12.9
Household margarine[2]	0.9	1.6	3.9	4.6	12.2
Bakery margarine[3]	0.5	2.2	4.4	6.2	11.8
Salad oil	0.6	1.6	4.3	5.0	13.0

[1] Fat acidity is expressed as ml. of 0.05 *N* NaOH required to neutralize the fatty acids in 2 g. of dough.
[2] Household margarine contained 45% of cocoa fat, 20% of rape seed oil, and 35% of hydrogenated whale oil.
[3] Bakery margarine was composed of 20% of rape seed oil, 30% of lard and tallow, and 50% of hydrogenated whale oil.

Rate of Lipolysis of Different Commercial Fats by Oat Flour

When the soapy taste in bread was first encountered, some thought that it was due to the nature of the shortening. Accordingly the relative susceptibility of different fats commonly used in Sweden to the lipase of unheated oat flour was investigated. The results in Table V show that all of the fats underwent extensive lipolysis, although the smell and taste of the doughs were different because of variations in the nature of the fatty acids which were liberated. These results support the view that the unpleasant taste experienced when certain oat flours are used in doughs is due to their high lipase activity.

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RELATION BETWEEN AMYLASE ACTIVITIES OF MALTED WHEATS AND MALTED WHEAT FLOURS [1]

W. F. GEDDES and P. P. MERRITT [2]

ABSTRACT

Malted wheats were readily milled on a two-stand micro experimental mill to a 95% patent flour representing a 50% yield by tempering the wheat to 13.5% moisture and employing a simple system of three breaks and four reductions. Differences in the amylase activity of the flour from replicate millings of one lot of malted wheat were statistically significant. The amylase activities of 30 malted wheats of widely varying activity bore a curvilinear relation to the activities of the flours milled from them, as determined by measuring the stimulation in dough gas production of a common base flour produced by 0.25% additions of each malt supplement. When amylase activity was measured in terms of the reciprocal of the dosage of the sample required to produce a given stimulation in gas production, there was

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[2] Present address: Red Star Yeast Company, Milwaukee, Wisconsin.

a linear relation between the corresponding values for malted wheat and malted wheat flours. The high correlation between these variables, $r = +0.984$, makes it possible to estimate the activity of the flours from the data for wheats with high accuracy, thus eliminating the labor and error involved in experimental milling.

In preparation for a further investigation of the effects of wheat variety, environment, and malting conditions on the properties of wheat malt (2), the present study was undertaken in 1942 to determine whether the value of malted wheat flours for breadmaking could be predicted from determinations on the malted wheats, thereby eliminating the labor and errors involved in experimental milling. At that time cereal chemical laboratories commonly evaluated malted wheat flour for breadmaking purposes by determining either the quantities required to give a definite flour maltose value or dough gas production (3, 7, 8). Hildebrand and Geddes (6) found that for any given malt the stimulation in gas production varied directly as the logarithm of the dosage, whereas the relationship between dosage and the increase in flour maltose value was best represented by a quadratic equation. The estimation of malt activity was thus more directly and simply made from gas production data than from flour maltose values. In applying the gas production technique they ascertained the dosage of malted wheat flour required to produce a given stimulation in gas production and expressed the activity in terms of the reciprocal of the required dosage.

This technique requires the addition of several levels of the malted wheat flour to a common base flour and, for greatest accuracy, it is necessary to estimate the dosage needed to produce the selected response from regression equations for the relation between malt flour dosage and gas production. Meredith, Eva, and Anderson (9) used a much less laborious technique in evaluating experimental malted wheat flours. This consisted in measuring the increase in gas production of doughs containing a standard flour which resulted from the addition of a fixed weight of malt flour.

In the present study, the amylase activities of ground malted wheats of widely varying activity were determined by the techniques of Hildebrand and Geddes (6) and of Meredith, Eva, and Anderson (9) and the values compared with those for the corresponding malted wheat flours produced by a simple experimental micro-milling procedure.

Materials and Methods

Wheat Malts. Thirty wheat malts, experimentally produced from soft white and hard red spring wheat during the course of an investigation by Dickson and Geddes (2), were employed. Fifteen malts were made from each wheat representing the combination of three steeping

levels (35, 40, and 45% moisture) and five germination times (2 to 6 days). These samples thus represented a wide range in amylase activity.

Milling. The milling was carried out in a two-stand micro mill similar to that described by Geddes and Frisell (4). Eighty-gram samples of each wheat malt were tempered to 12.5% moisture and allowed to stand for 16 to 18 hours; 30 minutes before milling, the moisture content was raised to 13.5%. Three breaks and four reductions were made with the separation of bran, shorts, 95% patent, and clear flour employing No. 18 wire, No. 64 GG, and No. 9 XX sieves. These three breaks were made without unloading the sieves which were shaken for 30 seconds after each break. The overs of the No. 18 wire sieve were discarded as bran. The middlings on No. 64 GG were reduced twice and the overs were discarded as shorts. The stock on the 9 XX silk was reduced twice and the overs weighed as clear flour. The throughs of each reduction were weighed separately and sufficient flour from the last reduction was added to that previously milled to obtain a 95% patent flour.

To secure an index of the replicability of the milling technique in respect to the amylase activity of the resulting flours, six samples from a bulk lot of malted wheat were milled by the foregoing procedure.

Amylase Activity. The amylase activities of the experimentally malted wheats and the corresponding flours were estimated in two ways, namely, by the technique of Davis and Tremain (1) as modified by Hildebrand and Geddes (6), and by making 0.25% additions of each to a common base flour of low gassing power and measuring the gas production of doughs prepared from them. The amylase activity of the flours obtained in the replicate millings of the commercial sample of malted wheat was determined only by the latter procedure. The malted wheats were ground in a laboratory model Wiley mill to pass a sieve having round holes 0.5 mm. in diameter. Moistures were determined on the ground wheats and flours and all dosages adjusted to a constant moisture basis. Duplicate doughs were made with 14 g. of each flour (including malt additions) and 10 ml. of yeast suspension containing 0.42 g. of compressed bakers' yeast; the gas production at 30°C. was measured over a 5-hour period.

Results and Discussion

Milling. Malting has such a pronounced mellowing effect that the malted wheats were very readily milled to an approximately 50% yield of 95% patent flour on the short system employed. For the six replicate millings of the commercial lot of malted wheat the standard error of patent flour yield was 0.74%. The mean gas production of

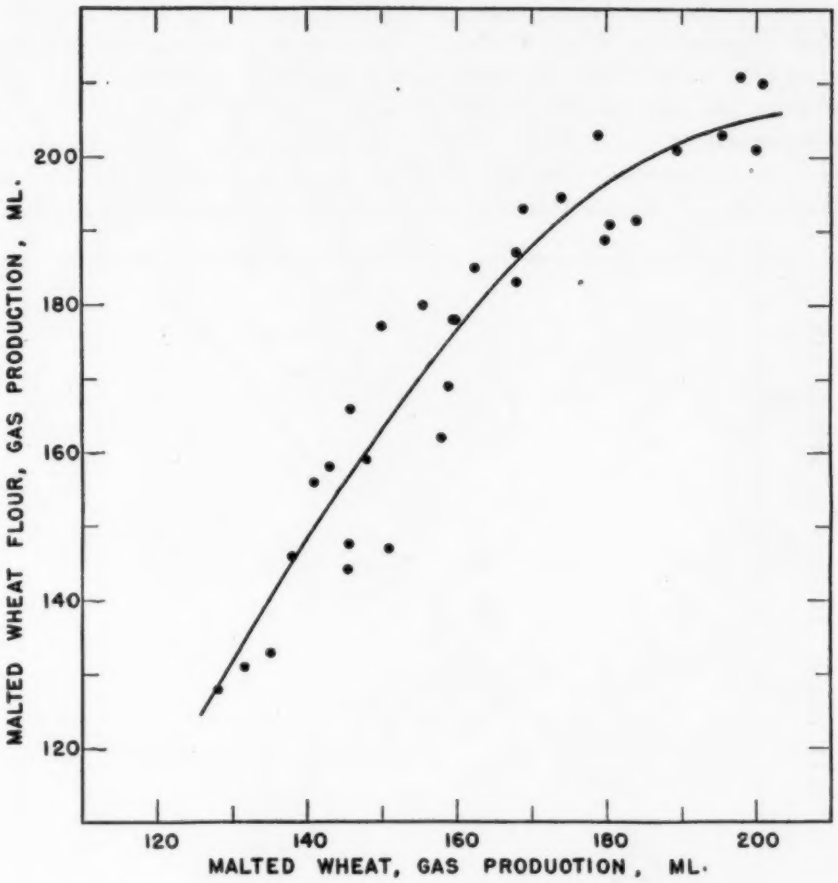

Fig. 1. Relation between the gas production of doughs supplemented with 0.25% additions of ground malted wheats and malted wheat flours milled therefrom. The curve was fitted by the method of least squares.

doughs containing 0.25% additions of these malted wheat flours varied from 198.6 to 202.7 ml. and a variance analysis showed that the differences due to milling were statistically significant.

Amylase Activity. The relation between the gas production of doughs supplemented with 0.25% additions of ground malted wheats and malted wheat flours, respectively, is shown in Fig. 1 and by the following statistical constants:

Statistics for gas production (30 pairs)	*Amylase supplement*	
	Malted wheat x	*Malted wheat flour* y
Mean, ml.	162.8	174.2
Standard deviation, ml.	21.5	24.2
Coefficient of variability, %	13.2	13.9

While the correlation based on the assumption of a linear relationship is quite high ($r = +0.924$, 1% pt. = 0.463), an analysis of variance (10) showed that the deviations from linear regression were significant and that the data were best represented by the second degree polynomial: $y = a + bx + cx^2$ where y and x represent the activities of malted wheat flour and malted wheat respectively and a, b, and c are constants. When allowance was made for curvilinearity of regression,

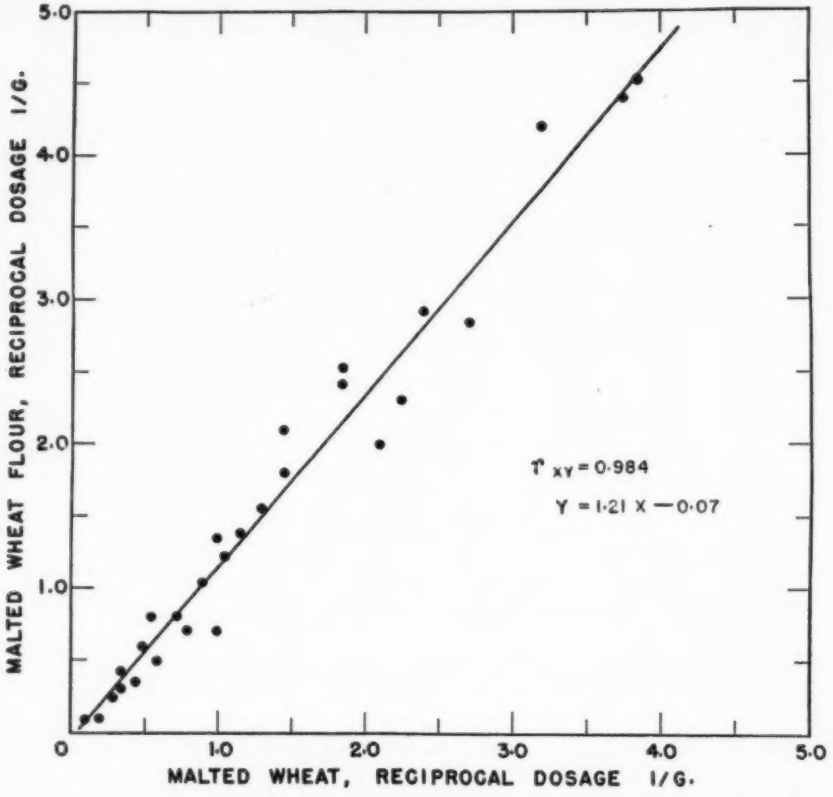

Fig. 2. Relation between the amylase activities of malted wheat and the flour milled therefrom, when the activity is measured in terms of the reciprocal of the dosage (grams supplement per 100 g. flour) required to produce a constant gas production in a wheat flour dough.

the total correlation between the gas production values was increased to $r = +0.976$.

The flours are more active than the malted wheats. When the gas production of the doughs supplemented with the wheats exceeds about 180 ml., the activities of the wheats do not appear to be as widely differentiated as those of the flours.

The amylase activities of the malted wheats and corresponding flours, as expressed by the reciprocal of the dosage in per cent required

to raise the 5-hour gas production for a flour of low gassing power to 205.0 ml., are plotted in Fig. 2. The data were analyzed statistically and certain of the pertinent constants follow:

	Amylase supplement	
Statistics for reciprocal dosage (29 pairs)	*Malted wheat* x	*Malted wheat flour* y
Mean amylase activity, 1/g.	1.325	1.532
Standard deviation, 1/g.	1.060	1.300
Correlation, r_{xy}	+0.984	
Regression equation	$Y=1.207\,x-0.067$	

The regression between the amylase activities of malted wheats and their flours is linear and the high correlation makes it possible to estimate the activity of the flours from the data for wheats with high accuracy. In this series the standard error of prediction is ± 0.17 reciprocal dosage units.

This investigation confirms observations in the literature that the use of a single dosage of an amylase supplement in gas production tests is not a particularly desirable procedure for measuring the relative amylase activity of a series of samples (1, 5, 6). As the quantity of amylase added is increased either by using larger dosages of a given malt or by adding the same quantities of malts of varying activity, the stimulation in gas production becomes less and less with each increment of amylase added, that is, the analytical values obtained by such measures bear a curvilinear relation to the activity of the sample. Even though the curvilinear relationship may be quantitatively defined, it appears that the evaluation of high activities would be somewhat less precise by the single dosage method than by a method which gives a linear relationship.

When the amylase activity is estimated by ascertaining the reciprocal dosages required to produce a given stimulation in gas production, the relation between the values for malted wheats and flours is linear and of a high order. Determinations of the activity of malted wheats by this technique will serve as a convenient and reliable measure of their value for the production of malted wheat flours for use in breadmaking. Analyses of the ground malted wheats eliminate the labor and errors involved in experimental milling.

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EFFECT OF WHEAT CLASS AND GERMINATION MOISTURE AND TIME ON MALT YIELD AND AMYLASE ACTIVITY OF MALTED WHEAT [1]

J. G. DICKSON [2] and W. F. GEDDES [3]

ABSTRACT

Composite samples of grades one and two hard red spring, hard red winter, soft red winter, and soft white wheats representing marketings of these samples and grades for the 1940 and 1941 crops, respectively, were each malted in duplicate at three moisture levels (approximately 35, 40, and 45%) and five germination times (2, 3, 4, 5, and 6 days) at 16°C. and data obtained for malting loss, cleaning loss, and amylase activity.

Malting loss (which comprised the losses in dry matter due to leaching in the steep water, respiration, and the removal of rootlets and sprouts in cleaning the dried malt) was influenced significantly by wheat class, year, moisture, and time of malting. Over-all moisture levels and germination periods, the wheat classes ranked in order of increasing mean malting loss for each year were: soft red winter, hard red spring, hard red winter, and soft white wheat. The malting losses increased when the wheats were malted at higher moistures or for longer times.

Cleaning loss, which represented the loss in dry matter by the removal of rootlets and sprouts from the dried malts and is a rough measure of growth, was influenced by the same factors as malting loss of which it forms a part. The increase in cleaning loss with increased germination time varied rather

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Based on cooperative investigations between the Minnesota and Wisconsin Agricultural Experiment Stations and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.
[2] Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, and Agent, Division of Cereal Crops and Diseases, Agricultural Research Administration, U. S. Department of Agriculture.
[3] Professor of Agricultural Biochemistry, Division of Agricultural Biochemistry, University of Minnesota.

widely and was relatively small at the 35% moisture level which was too low for good root and sprout growth.

The relative amylase activities of the malts, measured in terms of the reciprocal of the malt dosage required to give a desired level of gas production in a fermenting dough, was influenced significantly by wheat class and especially by the moisture and time of malting. For the two years and over-all malting conditions, soft white wheat yielded malt of the highest activity, followed by soft red winter, hard red winter, and hard red spring wheat in the order named. With an increase in the moisture content at which the samples were germinated, there was a greater increase in the amylase activity of the malts prepared from soft white and soft red winter wheats than of those from the hard red spring and hard red winter wheats.

Malting loss and amylase activity were highly and significantly correlated ($r = +0.710$). The relative influence of germination moisture on these variables indicates the use of moisture levels around 40% as the most economical, since the increased loss in malting at 45% was not compensated by a proportional increase in amylase activity.

The production of malted wheat flour for use in breadmaking has assumed wide commercial importance, but there have been few published studies on the effects of wheat type, environment, and malting conditions on the properties of wheat malt. In a preliminary study, Geddes, Hildebrand, and Anderson (2) investigated the effect of two levels of protein content, germination moisture, germination time, and kilning temperature on the value of malt prepared from hard red spring and amber durum wheat. Amylase activity, expressed as the reciprocal of the amount of malted wheat flour (experimentally milled from the malts) required to produce constant gassing power in blends with a common base flour, was influenced by all factors except kilning. Increasing the germination moisture from 40 to 44% and the germination time at 54°F. from 3 to 5 days and the use of hard red spring wheat rather than durum raised the amylase activity. Meredith, Eva, and Anderson (5) found that the amylase activities of malted wheat flours prepared from hard red spring wheat malts exhibited marked differences due both to the variety of wheat and points of growth. Kneen, Miller, and Sandstedt (4) applied the techniques for determining alpha- and beta-amylase activity developed in their laboratory to a study of the changes in amylase activity which occurred during the germination of hard red winter wheat at different temperatures in their laboratory. Although these studies were not conducted under malting conditions, they showed that both time and temperature of germination were important factors in the conversion of bound beta-amylase to the free form and in developing alpha-amylase activity.

The present investigation, which was conducted in 1942, represents a continuation of the preliminary study of Geddes, Hildebrand, and

Anderson (2) on the effect of wheat type, environment, and malting conditions on the value of wheat malt for increasing the gas production of doughs in breadmaking. Composite samples of the two highest grades of four market classes of United States' wheats for each of two crop years were each experimentally malted at three moisture levels for five germination times giving a total of 240 samples, and data were obtained for malting loss, cleaning loss, and amylase activity.

Materials and Methods

Composite samples of grades one and two hard red spring, hard red winter, soft red winter, and soft white wheats representing marketings of these wheat classes and grades for the 1940 and 1941 crops, respectively, were malted and the malts studied for amylase activity. The wheat lots represented composites of the official grading samples of the major receipts for these classes at the principal terminal markets.[4]

TABLE I

WHEAT CLASSES, GRADES, AND MOISTURE AND PROTEIN CONTENT OF THE EIGHT COMPOSITE SAMPLES OF THE 1940 AND 1941 CROP

Sample no.	Year grown	Class	Grade	Moisture content %	Protein content[1] %
1	1940	Soft red winter	2	12.4	10.5
2	1941	Soft red winter	1 and 2	8.6[2]	10.7
3	1940	Hard red winter	2	8.3[3]	13.2
4	1941	Hard red winter	2	9.9	12.7
5	1940	Hard red spring	1	12.2	13.2
6	1941	Hard red spring	1	11.3	12.0
7	1940	Soft white	2	12.3	8.7
8	1941	Soft white	2	10.4	8.0

[1] N × 5.7; expressed on 14% moisture basis.
[2] Stored at Wooster, Ohio, until just prior to malting.
[3] Stored at Manhattan, Kansas, until just prior to malting.
All other samples stored in unheated granary at Madison, Wisconsin.

The 1940 samples were stored under good conditions until the 1941 samples were received. The two seasons' wheats were cleaned and malted during the spring of 1942. The grade, moisture content, and protein content of the wheats are given in Table I.

Malting. The samples were malted in the Madison, Wisconsin, experimental malting unit at 16°C. (60.8°F.) at three moisture levels for 2, 3, 4, 5, and 6 days germination. The original, cleaned samples were divided through a Boerner divider into 60 approximately 200-g. lots for each class of wheat. Samples of 170 g., dry basis, were weighed out and stored until malted. Duplicate samples were malted as two independent series. The wheat classes for the two seasons were

[4] Samples were furnished by Robert H. Black, Grain Standards Research, Grain and Seed Division, Agricultural Marketing Service, United States Department of Agriculture.

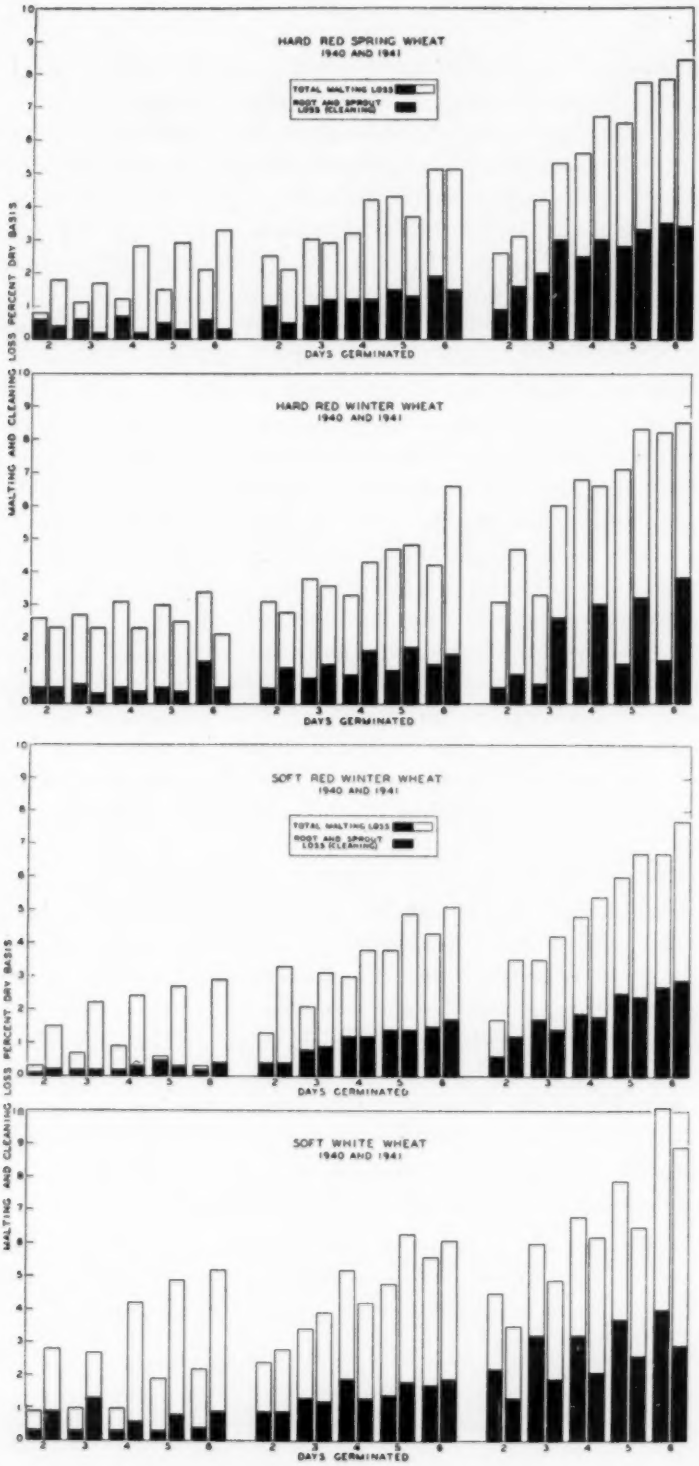
randomized within each duplicate series for each moisture level. The lots of five samples each for 2–6 day germinations were placed in the steep and malting chamber at intervals so that all samples went into the dryer at the same time. The germinated wheats were dried to about 12% moisture according to the following schedule: 8 hours at 25°C. (77°F.), 4 hours at 35°C. (95°F.), 12 hours at 45°C. (113°F.), and 5-7 hours at 55°–75°C. (131°–167°F.). After drying, the samples were weighed, cleaned to remove rootlets and sprouts, reweighed, and stored.

Amylase Activity. Since Geddes and Merritt (3) found that the amylase activity of ground malted wheats and of the flours prepared from them were highly correlated, amylase activity was determined on the malted wheats, thereby eliminating the labor and errors involved in experimental milling. The activity was determined in duplicate on each of the 240 malts, ground in a Wiley mill to pass the 0.5 mm. sieve, by adding increments to a low-diastatic flour measuring the gassing power, and computing the reciprocal of the dosage in per cent necessary to yield the same quantity of gas (205 ml.) as a flour possessing a maltose value of approximately 280 units.[5] Doughs prepared from 14 g. of flour (including the malt addition) on a 13.5% moisture basis and 3.0% yeast were fermented at 30°C. for 5 hours and the gas production measured volumetrically. The reciprocal of the malt dosage required to give the desired level of gas production was computed from the regression equation for the relation between gas production and the logarithm of the malt dosage (expressed in per cent).

Methods Used in Analysis of Data. The data on the influence of malting conditions on malting loss and amylase activity values of the four wheat classes were evaluated by several methods. Malting the four classes of wheat at three moisture levels and for periods of 2–6 days germination involved some modification of the usual procedures for evaluating data and drawing conclusions. The analysis of variance was used on the data in two ways.[6] First, an analysis of variance was made on the data using the mean values for the five germination periods; in other words, the effect of germination time was omitted by using the average of the data for the 2–6 day germination periods. Secondly, an analysis was conducted on the logarithmic transformation

[5] When these studies were undertaken, cereal chemical laboratories commonly evaluated malted wheat flour for breadmaking purposes by determining either the quantities required to give a definite maltose value or dough gas production. It is now generally agreed that the value of malt supplements for breadmaking depends on their alpha-amylase activities. Currently, amylase activities of doughs in excess of those required to maintain adequate sugar levels for the support of yeast fermentation are recommended because of their beneficial effects on bread quality. The favorable influence of amylase supplements on bread characteristics is related to the extent of starch modification by alpha-amylase during oven-baking and measurements of starch degradation during gelatinization have been proposed to replace those heretofore used.

[6] Credit is gladly given to Dr. C. H. Goulden, Cereal Division, Department of Agriculture, Ottawa, Ontario, Canada, and to Dr. J. H. Torrie, Department of Agronomy, University of Wisconsin, for assistance on this phase of the problem. Dr. Goulden suggested the logarithmic transformation of the data and applied the homogeneity test to verify the validity of the transformation on part of the data.


HARD RED SPRING WHEAT
1940 AND 1941
TOTAL MALTING LOSS
ROOT AND SPROUT LOSS (CLEANING)
MALTING AND CLEANING LOSS PERCENT DRY BASIS
DAYS GERMINATED
HARD RED WINTER WHEAT
1940 AND 1941
MALTING AND CLEANING LOSS PERCENT DRY BASIS
DAYS GERMINATED
SOFT RED WINTER WHEAT
1940 AND 1941
TOTAL MALTING LOSS
ROOT AND SPROUT LOSS (CLEANING)
MALTING AND CLEANING LOSS PERCENT DRY BASIS
DAYS GERMINATED
SOFT WHITE WHEAT
1940 AND 1941
MALTING AND CLEANING LOSS PERCENT DRY BASIS
DAYS GERMINATED

of the complete data. To avoid negative logarithms, 1.0 was added to all values. Obviously the first analysis gave an incomplete evaluation of the response of the wheat classes as it was impossible by this procedure to show the effect of time on the malting response. The latter method of analysis evaluates the influence of the various factors and the interaction between these factors and wheat classes and years in which the wheats were produced. Applying Bartlett's test (1) for homogeneity to the transformed data gave chi square values which were close to the 50% point so that the differences between the variances were about what would be expected on the basis of random sampling from a homogenous population.

Results and Discussion

The data and mean values for the four classes and two years for malting losses, cleaning losses, and amylase activity values are presented graphically. The data on malting and cleaning loss are presented by means of histograms in Fig. 1. The mean values for each of the three factors are given in Fig. 2. The influence of moisture levels in the steep and during malting and germination time on malting loss and amylase activity values using the mean values for the four wheat classes and two years is shown in Fig. 3. In constructing the graphs the moisture levels were arranged along one axis and germination time along the other. The functions of the moisture reactions at a given time form a series of lines perpendicular to a base plane common to both moisture level and germination time. The relations between moisture and time for the two factors, malting loss and amylase activity values, are shown for the entire moisture and time range. For clarity of presentation, the tops of the vertical lines representing reaction are connected forming a sectional surface or plateau, which

Fig. 1. Malting and cleaning losses for wheat composites grown in 1940 and 1941. The 1940 composites are represented by the histograms on the left of each pair; 1941 composites are on the right. The wheats were steeped at three moisture levels of approximately 35, 40, and 45% reading from left to right.

The actual moisture values were as follows:

Wheat class	*Approximate moisture* %	*Actual moisture 1940* %	*Actual moisture 1941* %
Hard Red Spring	35	33.5	35.2
	40	40.5	40.3
	45	45.5	45.8
Hard Red Winter	35	34.7	34.6
	40	40.0	40.6
	45	46.3	46.0
Soft Red Winter	35	32.3	34.4
	40	39.4	39.9
	45	45.2	45.8
Soft White	35	34.2	34.6
	40	40.5	41.0
	45	46.0	45.5

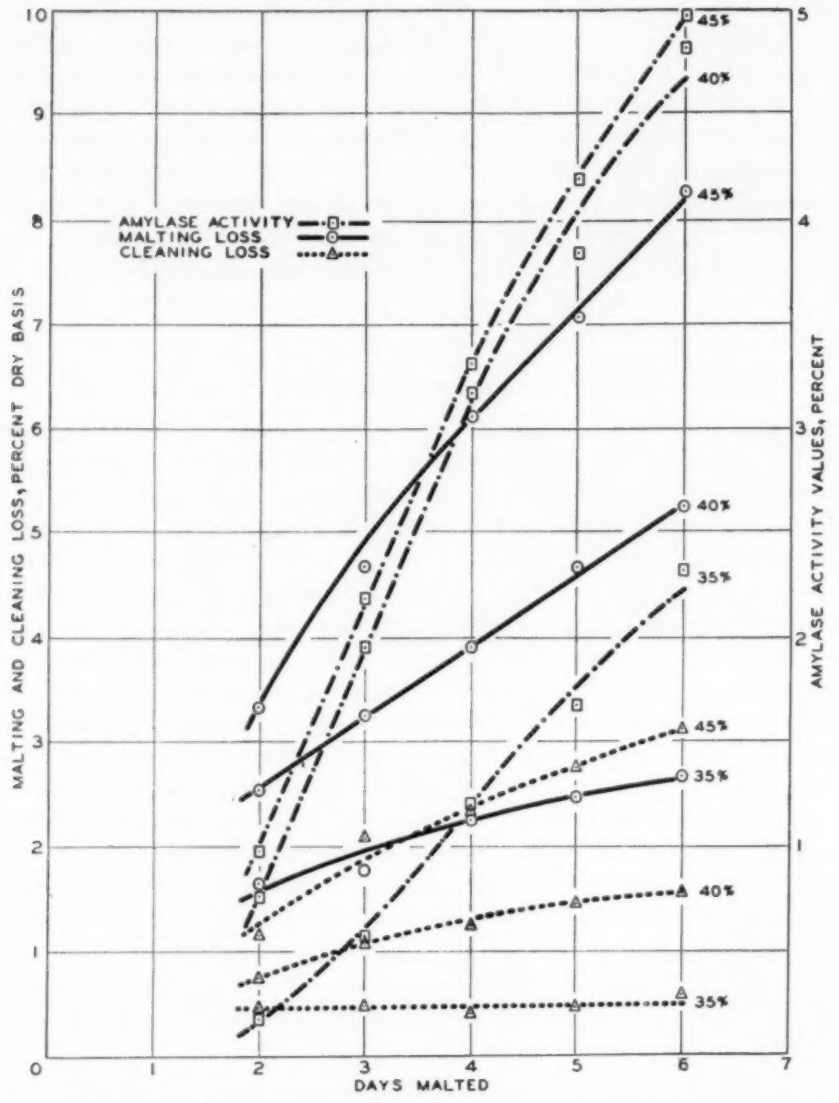


Fig. 2. The influence of moisture and time on malting and cleaning losses and amylase activity in wheat. The curves represent the mean values for the four classes and two years.

represents the interaction between moisture and time. The influence of moisture and time upon the reaction of the factor is shown by the rise in the plateau along the two axes. The reaction of the four wheat classes at the three moisture levels for malting loss and amylase activity values is given in Fig. 4. The construction of the graphs is similar to those in Fig. 3, except that moisture levels and wheat classes are arranged along the two axes. The mean values for 2–6 days germina-

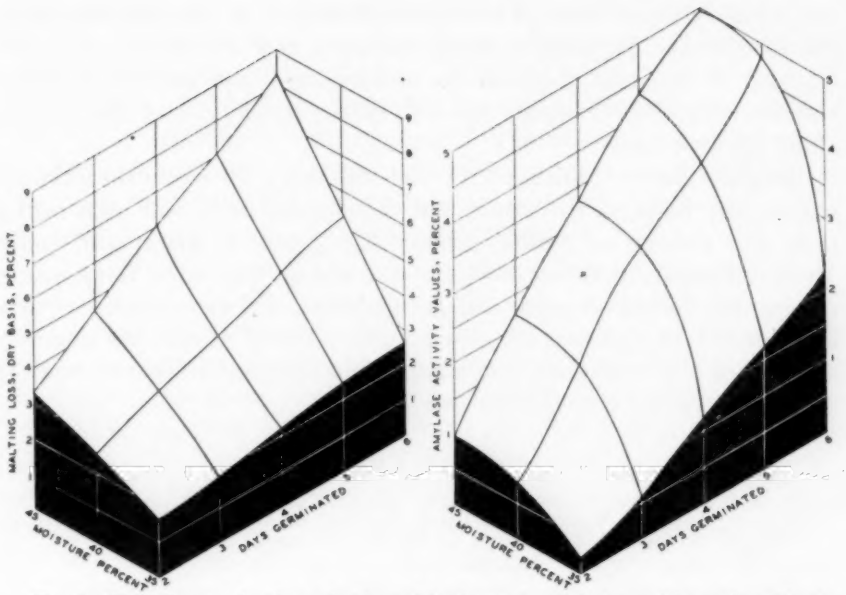


Fig. 3. The influence of moisture levels used in the steep and during malting and germination time in days on malting loss and "amylase activity" values using the mean values for the four wheat classes and the two years. Moisture levels are arranged along one axis and germination time along the other. The functions of the moisture reactions at a given time form a series of lines perpendicular to a base plane common to both moisture level and germination time. The tops of the vertical lines are connected forming a sectional surface or plateau which represents the interaction between moisture and time. The influence of moisture and time upon the factors is shown by the rise in the plateau along the two axes.

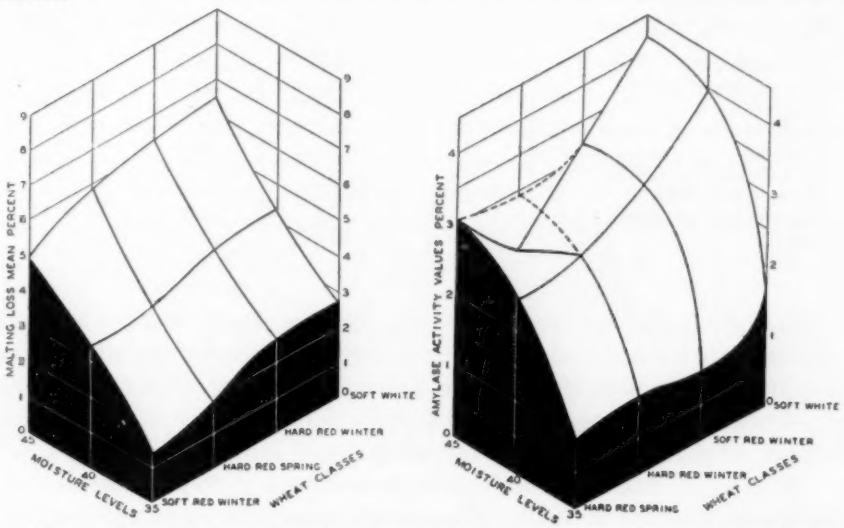


Fig. 4. The reaction of the four wheat classes malted at the three moisture levels for malting loss and amylase activity using the mean values for the five germination times and the two years. The construction of the graphs is similar to those in Fig. 3 except that moisture levels and wheat classes are arranged along the two axes. The sectional surface or plateau represents the interaction between malting moisture and wheat classes. The plateau surface represented by the broken lines is constructed on the basis of no interaction of the hard red winter at the 45% moisture level. The reaction of the wheat classes to malting loss and amylase activity at the three moisture levels is shown by the rise of the plateau along the two axes. The shift in rank of wheat classes for the two factors, malting loss and amylase activity, is shown.

tion periods were used. The sectional surface or plateau represents the interaction between malting moisture and wheat classes. The reaction of the wheat classes to malting loss and amylase activity values at the three moisture levels is shown by the rise of the plateau along the two axes.

Malting Loss. Malting loss, the difference between the original weight, dry basis, of the wheat and the cleaned malt, was used rather than the volume or bushel relationship between wheat and malt. Malting loss included loss in weight due to leaching in the steep water, respiration during steeping and germination, and removal of rootlets and sprouts in cleaning the dried malt. The *F* values obtained by analysis of variance using the logarithmic transformation of the data for malting loss are given in Table II.

TABLE II

ANALYSIS OF VARIANCE OF DATA FOR MALTING LOSS, CLEANING LOSS, AND AMYLASE ACTIVITY VALUES FOR FOUR CLASSES OF WHEAT GROWN IN TWO YEARS MALTED IN DUPLICATE AT THREE MOISTURE LEVELS FOR FIVE GERMINATION TIMES

Source of variation	Degrees of freedom	Mean squares		
		Malting loss	Cleaning loss	Amylase activity values
Wheat classes	3	.198**	.092**	.272**
Years	1	.476**	.040**	.001
Moisture levels	2	2.632**	2.200**	2.019**
Germination time	4	.445**	.189**	1.742**
Wheat × years	3	.078**	.055**	.104**
Wheat × moisture levels	6	.019	.031**	.043**
Wheat × germ. time	12	.005	.005	.006
Years × moisture levels	2	.105**	.030**	.015
Years × germ. time	4	.002	.001	.009
Moisture levels × germ. time	8	.026	.042**	.018
Error	120	.016	.005	.012

Based on the two years' samples, malting losses were influenced significantly by wheat class, year, moisture, and time of malting. The wheat classes ranked in ascending order for the mean malting loss of all moisture levels and germination periods for each year were: soft red winter, hard red spring, hard red winter, and soft white wheat.

The malting losses were higher generally for the 1941 wheats than for those collected in 1940. The wheat classes varied in malting loss within the two years sampled and they responded differently when malted at different moisture levels (Table II). While there was considerable variation between duplicates, the upward trends for the increases due to longer germination times and higher moistures were

highly significant. The curves for comparative mean malting loss for the four classes and two years combined, Figs. 2 and 3, indicate the relationships to moisture and time.

Cleaning Loss. In cleaning the dried wheat malts, the rootlets and many of the sprouts were removed. The cleaning loss represented the weight of rootlets and sprouts removed and was a rough, comparative measure of growth. Cleaning loss was included in the malting loss discussed previously (Figs. 1 and 2 and Table II).

Cleaning loss was influenced by wheat classes, years, moisture levels, and germination time, as in the case of malting loss. Significant interactions for wheats × years and years × moisture levels were again similar to malting loss. In addition, significant interactions for wheats × moisture levels and moisture levels × germination time occurred in cleaning loss. In the hard red winter class for 1940 the cleaning loss for samples malted at 45% moisture was lower than for those malted at 40% moisture. The increase in cleaning loss with increased germination time varied considerably, especially at the 35% moisture level where moisture was too low for good root and sprout growth.

Amylase Activity. The amylase activities of the malts were influenced significantly by wheat class and especially by the moisture and time of malting. Significant interactions were found for wheats × years and for wheats × moisture levels. For the two years and over-all malting conditions, soft white wheat yielded malt of the highest activity followed by soft red winter, hard red winter, and hard red spring wheat in the order named. The interaction of years by wheat classes is due largely to a change in the relative ranking of hard red spring and hard red winter wheats. For 1940 the activities of their malts fell in third and fourth place, respectively, while the reverse was the case for the 1941 samples. With an increase in the moisture content at which the samples were germinated, there was a greater increase in the amylase activity of the malts prepared from soft white and soft red winter wheats than of those from the hard red spring and hard red winter wheats.

The contrast between the shape of the plateaus (Figs. 3 and 4) for malting loss and amylase activity values suggests a linear relation in the former and a quadratic relation in the latter. Tests for linear and quadratic functions of the two sets of data show this to be the case.

Simple correlations were computed between the data on malting loss and amylase activity values. The positive correlation ($r = +0.710$) between the two factors was highly significant, indicating that in these wheat classes for the two years sampled, malting loss might be used as an index of the relative amylase activity to be

expected. In practical application malting loss can be evaluated against amylase activity.

The relative influence of moisture level on malting loss and amylase activity values, Fig. 4, suggests the use of moisture levels around 40% as the most economical for wheat malting. The increased loss in dry weight in malting at 45% moisture in contrast with the loss at 40% was not compensated by a proportional increase in amylase activity. This relationship was indicated for all four classes of wheat.

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EFFECT OF WHEAT CLASS AND MALTING CONDITIONS ON GASSING-POWER STIMULATION AND ALPHA- AND BETA-AMYLASE ACTIVITIES OF MALTED WHEAT [1]

W. F. GEDDES,[2] J. G. DICKSON,[3] and C. B. CROSTON [4]

ABSTRACT

Nineteen composite samples of wheat malts representing four wheat classes and variations in germination moisture and time were analyzed for free and total alpha- and beta-amylase activities. The activities, expressed as a reciprocal of the percentage addition of each malt required to produce a given stimulation in the gas production of a fermenting dough, were also ascertained. The free and total alpha-amylase values agreed closely and increased in parallel fashion with increases in germination moisture and time. In contrast, the total beta-amylase was virtually constant for all malts, but as germination progressed, there was a marked increase in free beta-amylase. The various positive correlations between the different measures of amylase activity were all of a very high order of magnitude.

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[2] Professor of Agricultural Biochemistry, Division of Agricultural Biochemistry, University of Minnesota.

[3] Professor of Plant Pathology, Department of Plant Pathology, University of Wisconsin, and Agent, Division of Cereal Crops and Diseases, Agricultural Research Administration, U. S. Department of Agriculture.

[4] Research Assistant, Division of Agricultural Biochemistry, University of Minnesota. Present address: Northern Regional Research Laboratory, Peoria, Illinois.

The partial correlation between free alpha-amylase activity and the reciprocal of the malt dosage required to give a uniform gas stimulation, independent of free beta-amylase activity, was +0.92, in contrast to a low and insignificant correlation of +0.14 between beta-amylase activity and reciprocal malt dosage, independent of free alpha-amylase activity. These results support the widely held view that the alpha-amylase activity of malts is the important factor in relation to their stimulating effect on the gas production of fermenting doughs.

The wheat malts prepared by Dickson and Geddes (1) in their investigation of the effect of wheat class and malting conditions on the value of the malts for increasing the gas production of wheat flour doughs provided valuable material for a study of the development of free and total alpha- and beta-amylase activities at various stages of the malting process and the relation of these activities to gas stimulation in doughs. Since Kneen, Miller, and Sandstedt (2) had just reported a comprehensive investigation of the changes in amylase activity that occurred during the germination of hard red winter wheat at various temperatures, the present study was limited to a series of 19 composites representing the different wheat classes, germination moistures, and germination times.

Materials and Methods

Nineteen composites were prepared from equal weights of certain of the individual ground malts described by Dickson and Geddes (1). One composite was prepared for each of the three germination moistures (approximately 35, 40, and 45%) and for each of five germination times (2 to 6 days inclusive) to give a total of 15 composite samples, each of which contained 16 individual malts representing duplicate maltings for each of two crop years of hard red spring, hard red winter, soft red, and soft white wheats. Another set of four composites was prepared which represented the four respective wheat classes; in this series, each composite contained 24 individual malts, which represented duplicate maltings for the two crop years at two moisture levels (approximately 40 and 45%) and three germination times (4, 5, and 6 days).

The techniques used by Kneen, Miller, and Sandstedt (2) in a similar study were followed, with certain minor modifications. Preliminary experiments showed that the dextrinogenic activities of extracts made with 0.1 and 0.2% calcium acetate solution were very similar and somewhat higher than corresponding extracts made with distilled water. As 0.1% calcium acetate extracts gave beta-amylase values in close agreement with those for distilled water extracts, there was no evidence of an inactivating effect (5) of calcium ions at this concentration; accordingly, both the free and total extracts were made

by extracting 1.0 g. of malt with 100 ml. of 0.1% calcium acetate solution. This ratio of malt to liquid was used to avoid subsequent dilution, or the use of less than 1.0 ml. of the more highly active extracts, in the dextrinogenic determinations. A two-hour extraction time at 30°C. was used in preparing the free extract, whereas the total extract was obtained by employing 0.5 g. of papain (Parke, Davis and Company) per gram of malt with an extraction time of 18 hours at 30°C. Preliminary trials showed that this quantity of papain was required to ensure maximum total saccharogenic activity.

The alpha- and beta-amylase activities of the malt extracts were determined by the respective methods of Sandstedt, Kneen, and Blish (7) and of Kneen and Sandstedt (3).

Results and Discussion

The results are summarized in Table I together with the corresponding mean amylase activities determined by ascertaining the reciprocal dosage of malt required to produce a given stimulation in the gas production of a fermenting dough. These mean amylase units were computed from the appropriate data obtained by Dickson and Geddes (1) for the individual malts. The correlation coefficients involving these different measures are given at the foot of the table.

The data for these malts which were prepared under conditions similar to those used in commercial malting confirm the general trends noted by Kneen *et al.* (2) who used a "rag doll" technique for malting. The free and total alpha-amylase values are in close agreement and increase in parallel fashion with increases in germination moisture and time. In contrast, the total beta-amylase is virtually constant for all the malts, but as germination progresses there is a marked increase in free beta-amylase. However, only approximately 50% of the total beta-amylase was present in the free condition even after germinating wheat containing 45% moisture for six days (at 16°C.). This is not in agreement with the results obtained by Kneen *et al.* who found that the free and total activities became essentially equal during the later stages of germination, nor is it in accord with the experience with barley malts. Wheat class appears to have some influence on the percentage of the total beta-amylase which is present in the free state under corresponding malting conditions, but one would hardly expect the class or variety of wheat to be the complete explanation for these differences. Variations in the severity of the heat treatment employed in drying the malts in different studies would influence their amylase activities.

The various correlations between free alpha-amylase, total alpha-amylase, free beta-amylase, and amylase activity determined by the

gas production technique are all of a high order of magnitude. The correlation of +0.984 between free (as well as total) alpha-amylase activity and the activity measured by the gas stimulation method

TABLE I

EFFECT OF MALTING CONDITIONS AND WHEAT CLASS UPON AMYLASE ACTIVITIES

Nature of sample / Germination conditions		Alpha-amylase[1]			Beta-amylase[2]			Amylase activity from gas production[3]
Approx. moisture %	Time days	Free	Total	Free as % total	Free	Total	Free as % total	1/g.
Based on Composite Malts from Four Wheat Classes[4]								
35	2	3.8	4.6	83	4.5	19.6	23	0.10
	3	9.3	10.5	88	4.9	19.7	25	0.27
	4	17.9	21.2	84	5.4	20.3	27	0.57
	5	28.8	30.8	93	6.6	20.4	32	0.80
	6	43.8	42.0	104	7.6	20.6	37	1.11
40	2	13.6	15.3	89	4.6	20.1	23	0.38
	3	36.6	36.7	100	6.0	20.6	29	0.99
	4	58.6	58.6	100	7.8	20.5	38	1.58
	5	68.5	71.9	95	9.1	20.5	44	2.24
	6	89.6	91.0	98	10.1	20.5	49	2.41
45	2	19.0	19.0	100	6.7	19.8	34	0.50
	3	39.8	39.6	100	8.3	20.2	41	1.09
	4	61.4	65.2	94	9.5	20.4	47	1.65
	5	76.1	75.3	101	10.2	20.4	50	2.15
	6	91.4	96.1	95	10.3	21.0	49	2.48
Based on Composite Malts Germinated at Approximately 40 and 45% Moisture for 4, 5, and 6 days								
Wheat class								
Soft white		77.3	79.6	97	9.6	15.2	63	2.65
Soft red winter		71.9	74.4	97	12.8	22.1	58	2.22
Hard red spring		64.8	71.3	91	10.1	22.4	45	1.83
Hard red winter		56.8	62.9	90	8.2	19.2	43	1.64

Correlation Coefficients

Free alpha-amylase × amylase activity (gas production) = +0.984
Total alpha-amylase × amylase activity (gas production) = +0.984
Free beta-amylase × amylase activity (gas production) = +0.902
Free alpha-amylase × total alpha-amylase = +0.997
Free alpha-amylase × free beta-amylase = +0.905
Total alpha-amylase × free beta-amylase = +0.904
(Value of r at the 1% point = 0.575.)

[1] The alpha-amylase unit is the number of grams of soluble starch which is dextrinized by the alpha-amylase of 1.0 g. malt (dry basis) in 1.0 hour at 30°C. in the presence of excess beta-amylase.
[2] The beta-amylase unit is the number of grams of soluble starch converted to maltose by the beta-amylase of 1.0 g. malt (dry basis) in 1.0 hour at 30°C.
[3] Reciprocal dosage of malt required to produce a given stimulation in gas production.
[4] The beta-amylase activity of a composite sample of the four wheat classes was: free beta-amylase = 3.8 units; total beta-amylase = 20.0 units; free as % of total = 19%.

indicates that the increased gassing power of a dough upon the addition of malted wheat meal is almost entirely dependent on alpha-amylase activity. This supports the conclusion of Kneen and Sandstedt (4) that determinations of alpha-amylase and increased gassing power "appear to be equally reliable for evaluating malts." These workers obtained a correlation of 0.94 between these two measures. On the other hand, Meredith *et al.* (6), employing malt flours milled from 39 malted wheats prepared from composite samples of wheat representing several hard red spring wheat varieties and a number of locations in western Canada, obtained correlations of 0.71 for varieties and 0.79 for stations. They point out that a close association between two such properties in a series of samples which are produced by progressively changing the processing methods does not necessarily indicate a causal relation.

In the present study free beta-amylase and amylase activity by the gas production method were highly correlated (+0.902); as the correlation between free alpha- and free beta-amylase is also quite high, the simple correlation coefficients do not give a measure of the relative importance of alpha- and beta-amylase in relation to the stimulation of gas production in doughs. The partial correlation between free alpha-amylase activity and the reciprocal of the dosages of malt required to give a uniform gas stimulation, independent of free beta-amylase activity, is +0.916, whereas the corresponding correlation of +0.144 between beta-amylase activity and reciprocal malt dosage, independent of free alpha-amylase activity, is not statistically significant. These results, therefore, support the widely held view that the alpha-amylase activity of malts is the important factor in the stimulation of gas production which is obtained when malt products are added to fermenting doughs.

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COMMUNICATION TO THE EDITOR

A Method for Staining Insect Egg Plugs in Wheat

DEAR SIR:

With the attention now being given to mill sanitation, it is becoming increasingly necessary that a more satisfactory method be developed for the identification of weevil infestation in wheat. The use of stains, such as acid fuchsin and iodine-potassium iodide, has been reported, but they have the disadvantage of staining exposed endosperm as well as weevil egg plugs. The gentian violet procedure given below is a selective stain for insect egg plugs and, under the conditions described, will not stain the endosperm of the wheat kernel.

The wheat sample (usually 5 g.) is steeped in warm water containing a wetting agent. Three drops of Triton W-30 (a sulfated aromatic ether alcohol; Rohm and Haas Co., Inc.) in 200 ml. of water will satisfactorily wet the wheat in approximately 30 seconds. Other wetting agents, such as Aerosol OT (dioctyl sodium sulfosuccinate; American Cyanamid Co.) and Sterox SK (a polyoxyethylene thioether; Monsanto Chemical Co.), also are satisfactory. The sample is easily handled if it is placed in a small wire container or in a container with a wire bottom so that it can be transferred from one solution to another. When the sample is removed from the wetting solution, the excess water should be removed by placing the container with the wet wheat, or the wet wheat alone, on a dry towel for a few seconds before putting the sample into the staining solution.

The wheat is exposed for 2 minutes in a solution which contains 10 drops of a 1% aqueous stock solution of gentian violet (Coleman-Bell Co.) in 50 ml. of 95% ethanol. The sample then is removed from the staining reagent and washed in clear water for approximately 20 seconds, or until the excess stain held by the brush end of the kernel is removed. The insect egg plugs are a purple color and are very easily distinguished at 5 to 7 magnifications. The egg plugs are seen most clearly while the kernels are wet or if the sample is placed in water.

Good results are obtained by this gentian violet staining technique, but there are some precautions that should be observed. The insect egg plugs in wheat at the usual moisture content will not stain in an alcoholic solution unless the kernels are first wet with water. On the other hand, if the excess water is not removed from the wetted sample, the wheat kernel will stain completely. Also, if the wheat is badly

damaged and the bran is torn or cracked, one of the under layers of the pericarp will stain around the edge of the tear. This, however, will not be confused with the insect egg plugs nor will it obscure or prevent any egg plugs from staining.

Although the aqueous stock solution has a long storage life, the efficiency of the dilute alcoholic stain becomes unsatisfactory when the solution takes on enough water to stain or tint the entire wheat kernel. The number of samples that can be stained in one solution depends upon the wetness of the wheat placed in this alcoholic solution.

This gentian violet staining procedure, when the above-mentioned precautions are observed, gives more satisfactory results than other procedures.

July 6, 1949

HAROLD J. GOOSSENS
Russell-Miller Milling Company
Minneapolis, Minnesota

BOOK REVIEWS

Advances in Food Research. Volume I. Edited by E. M. Mrak and George F. Stewart. 459 pp. Academic Press Inc., New York, N. Y. 1948. Price $7.50.

The book contains 10 chapters dealing with various phases of Food Chemistry. Bate-Smith from Cambridge, England, gives an excellent review of the present status of knowledge concerning the physiology and chemistry of rigor mortis with special reference to the aging of beef. This article should be of special interest to chemists in the meat packing industry since it relates the changes occurring during rigor mortis to the glycogen content, the pH, the color of the flesh, the rate of penetration of salt during curing, the amount of drip from frozen meats after thawing, and the effects these factors have on the increase in tenderness during the ripening process.

Clifcorn of the Continental Can Company, Chicago, gives a detailed review of the factors influencing the vitamin content of canned foods. This review is of particular interest to people in the canning industry and in the field of nutrition. In various tables he gives the vitamin content of a variety of different kinds of canned foods and shows how these may be affected by the canning operations, by storage, and by the processes used preparatory to the serving of the foods.

Lepovsky from the University of California at Berkeley has a very interesting chapter on the physiological basis of voluntary food intake. He discusses such questions as whether or not humans and animals can choose foods wisely in accordance with their nutritional needs, the effect of proteins, amino acids, water-soluble vitamins, fats, and fat-soluble vitamins and minerals on the voluntary food intake. The factors influencing voluntary food intake are governed by very complex physiological mechanisms that are not too well understood, but the author gives a very good review of our present state of knowledge.

Lightbody and Fevold of the Quartermaster Food and Container Institute, Chicago, give a detailed discussion of the biochemical factors influencing the shelf life of dried whole eggs and means for their control. This article should be of extreme importance to those chemists who are concerned with the preparation of dried egg products. A great deal of research was carried out during the recent war on the factors responsible for the deterioration in quality of dried egg products during drying and storage. The authors give an excellent review of this research and bring out the factors that must be controlled if a good quality powder is to be produced and one that will retain its qualities during storage.

Miss Lowe of Iowa State College, Ames, Iowa, discusses the factors affecting the palatability of poultry products. She discusses the various factors that can influence quality with particular reference to those which can be controlled during processing.

Some excellent photomicrographs are included that show the structure of the protein and the changes that take place in the structure during aging.

Ross, Cornell University, Ithaca, New York, has an excellent review on the deterioration of processed potatoes. The article deals primarily with the problems involved in the production of dehydrated potatoes and shows what types of deterioration can take place in this product. The browning reaction receives the bulk of attention and the author discusses in detail various factors that contribute to browning and how it can be controlled.

Somers and Beeson discuss the influence of climate and fertilizer practices upon the vitamin and mineral content of vegetables.

Stadtman, University of California, Berkeley, has a comprehensive article on the nonenzymatic browning in fruit products. This is an excellent review of the theories and the effects of the various environmental factors on the browning reaction. After reading this article one appreciates the fact that the browning reaction is indeed complex and that a thorough understanding is necessary for proper control.

Wyss of the University of Texas, Austin, Texas, has a short review on the microbial inhibition by food preservatives. This article is not a catalogue of the microbial inhibitors that are used in foods but rather an academic treatment of the topic.

Baker, University of Delaware, Newark, discusses high-polymer pectins and their deesterification. This is a concise treatment of the topic, including the composition, source of materials, extraction methods, and methods of deesterification. He also indicates the properties and uses of the low ester pectins.

This is the first volume of *Advances in Food Research*. This volume should be an extremely valuable book for all food technologists, and if future volumes in this series continue in the same high level of quality as exhibited in the first volume, this series will make valuable reference books in the library of all food technologists. Dr. Mrak and Dr. Stewart are to be congratulated on a job well done.

H. O. HALVORSON
Dept. of Bacteriology & Immunology
University of Minnesota
Minneapolis, Minnesota

Sugar. By Andrew Van Hook. 155 pp. illus. Ronald Press Company, New York. 1949. Price $3.00.

This book should meet the needs of the reader interested in the nature and production of sugar (sucrose). Technical details necessary to an understanding of the formation of sugar in the plant, manufacture of the finished sugar, the place of sugar in world trade, and the uses of by-products have been well presented so that the average reader gains a good understanding of all phases of sugar production.

The book is made up of six chapters. Chapter I, an introduction, deals with the structure of matter, chemistry, particularly as related to sugar, and finally the uses of sugar. This is the least interesting chapter of the book, but doubtless will be useful to some readers.

Chapter II discusses the production of sugar cane and the cane sugar refined from it, with greatest emphasis upon factory processing. The various steps in factory processing have been dealt with in a brief but clear fashion. The reader should have no difficulty in grasping the fundamentals if not the details of cane sugar processing.

Chapter III deals with the production of sugar beets and beet sugar in much the same manner that the previous chapter deals with cane. Again the discussion is brief, but adequate.

Chapter IV, Commercial and Trade Aspects, discusses world production and consumption of sugar, as well as production by countries. The statistical data presented appear to be accurate and up to date.

Chapter V takes up the problem of by-products, cane bagasse, beet pulp, molasses, and possible uses of sugar in the production of chemicals. Use of sugar beet tops, a valuable farm by-product, is not discussed.

Chapter VI, History, should be of considerable interest to the reader who is interested in the growth and development of the sugar industry to its present status.

The book is well illustrated and indexed and the format is excellent.

J. O. CULBERTSON
Division of Agronomy
University of Minnesota
St. Paul, Minnesota

SUGGESTIONS TO AUTHORS

General. Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, all original drawings or photographs for figures, and one set of small photographic reproductions of figures.

Issues of *Cereal Chemistry* published after January 1, 1948, when some changes were made, are the most useful guides to acceptable arrangements and styling of papers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The institution from which the paper is submitted, author's connections, etc., are given in footnotes.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. Long introductory reviews should be avoided, especially when a recent review in a monograph or another paper can be cited instead. References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a series of separate studies is often best described with main sections for each study. Headings should be restricted to center headings for main sections and run-in italicized headings for subsections.

Tables. Data should be condensed and arranged to facilitate the comparisons the reader must make. Tables should be kept reasonably small by breaking up large ones, omitting all unessential data, and minimizing number of significant figures. Leader tables without number, main heading, or ruled lines are useful for small groups of data. Textual matter in tables should be minimized and unnecessary footnotes should be avoided.

Tables should be typed on separate pages at the end of the manuscript and their positions should be indicated to the printer by typing "(Table I)" in the appropriate place between lines of the text. (Figures are treated similarly.)

Figures. If possible, all drawings should be made by a competent draughtsman. Curves should be drawn heaviest, axes or frame intermediate, and grid lines lightest. The horizontal axis should be used for the independent variable; and experimental points should be shown. Labels are preferable to legends. All drawings should be made two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper. Lettering should be done with a guide, and letters should be $\frac{1}{16}$ to $\frac{1}{8}$ inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

Figures should be identified by lightly writing number, author, and title on the back. Cut-lines (captions) should be typed on a separate sheet attached to the end of the manuscript.

Style. Clarity and conciseness are the prime essentials of a good scientific style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*.

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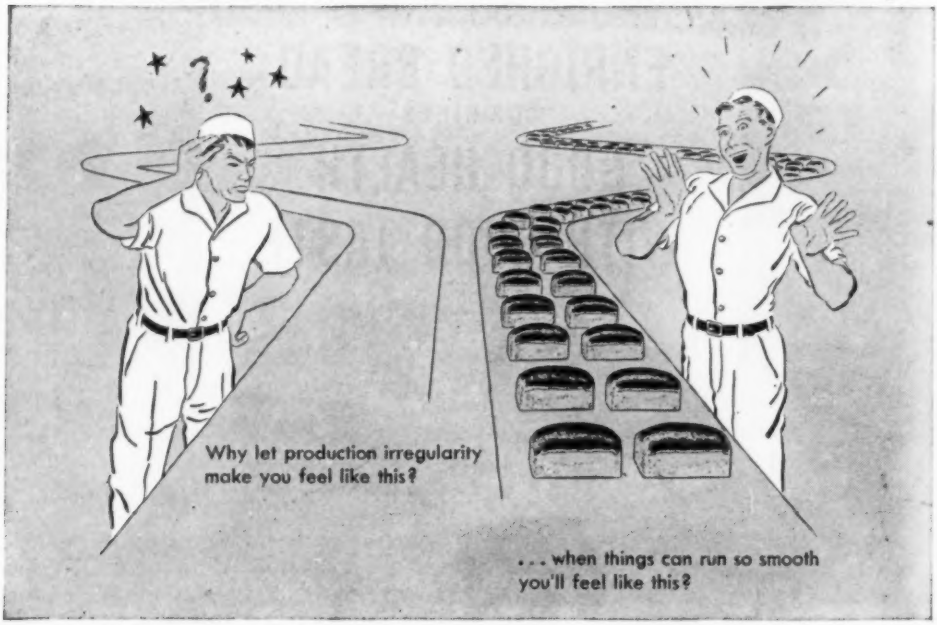

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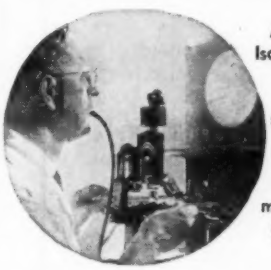

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